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UNDERWATER BONDING WITH POLYMER MIMICS OF MUSSEL ADHESIVE PROTEINS

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UNDERWATER BONDING WITH POLYMER MIMICS OF MUSSEL ADHESIVE PROTEINS

A Dissertation

Submitted to the Faculty

of

Purdue University

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Michael A. North

In Partial Fulfillment of the

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of

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A Ph.D is not done alone. There are countless individuals who help along the way. For me, first and foremost of these individuals are my maternal grandparents. Without their unwavering faith and belief in education I would not be where I am today. The memories and times I had with them are some of my most cherished, I am honored that I had the privilege of calling them family. Equally important is the love and support from my mother. She has supported me from the beginning and given up much so that I can be where I am today.

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ABBREVIATIONS

Mfp	Mytilus edulis foot protein
DOPA	3,4-dihydroxyphenylalanine
g	gram
cm	centimeter
mL	milliliter
N	newtons
MPa	megapascal
PVC	poly(vinyl chloride)
PTFE	poly(tetrafluoroethylene)
n-BuLi	*n*-Butyllithium
BBr3	boron tribromide
AIBN	2,2′-azobis(2-methylpropionitrile)
DCM	dichloromethane
MeOH	methanol
$CaCO_3$	calcium carbonate
Periodate	tetrabutyl ammonium periodate
TBAF	tetrabutylammonium fluoride
TFA	trifluoroacetic acid

NMR	nuclear magnetic resonance
^{1}H NMR	proton nuclear magnetic resonance
GPC	gel permeation chromatography
DSC	differential scanning calorimetry
M_n	number average molecular weight
M_w	weight average molecular weight
PDI	polydispersity index
TMSI	trimethylsilyl iodide
NaSEt	sodium ethanethiolate
DMSO	dimethyl sulfoxide
TPFPB	tris-(pentaflurophenyl)borane
NH_4F	ammonium flouride

ABSTRACT

North, Michael A. Ph.D, Purdue University, December, 2016. Underwater Bonding with Polymer Mimics of Mussel Adhesive Proteins. Major Professor: Jonathan J. Wilker.

When it comes to underwater adhesion, shellfish are the true experts. Mussels, barnacles, and oysters attach to rocks with apparent ease. Yet our man-made glues often fail miserably when trying to stick in wet environments. Results described herein focus on poly[(3,4-dihydroxystyrene)-*co*-styrene], a polymer mimic of mussel adhesive proteins. Underwater bonding was examined as a function of several parameters including polymer molecular weight and composition. In doing so, several surprising results emerged. Poly[(3,4-dihydroxystyrene)-*co*-styrene] may be the strongest underwater adhesive found to date. Bonding even exceeded that of the reference biological system, live mussels. Adhesion was also found to be stronger under salt water versus deionized water. Such unexpected findings may contradict earlier proposals in which charged amino acids were thought to be key for mussel adhesive function. Taken together, these discoveries are helping us to both understand biological adhesion as well as develop new materials with properties not accessed previously.

Reducing the mussel adhesive proteins to the simplest level revealed exciting results for underwater adhesion. Building off of this success, additional components of the mussel adhesive system where selected to be incorporated into the polymer mimic. Charged groups have been incorporated into poly[(3,4-dihydroxystyrene)-*co*-styrene]

before, however, the route was six steps that involved multiple protection/deprotection steps. This synthetic burden has been reduced to three steps with the final step being still being optimized for complete deprotection.

Having achieved significant bonding in underwater environments with poly[(3,4-dihydroxystyrene)-*co*-styrene] attempts were made to bring this system out of the laboratory and into the real world. Drawing inspiration from existing commercial products in addition to mussels and squids a delivery system was designed and tested which would allow for better commercial applicability. Testing has revealed that formulating poly[(3,4-dihydroxystyrene)-*co*-styrene] for commercial delivery will require several hurdles to be overcome and the groundwork has been well established for further study.

CHAPTER 1. MUSSEL BIOMIMETIC ADHESIVES

1.1. Adhesives: The Return to Nature

Throughout the ages mankind has used adhesives. The earliest known examples of adhesives being used, date back to ~ 200,000 years ago (*1*), where an adhesive composed of tar and pitch was used to stick together two pieces of a rock tool. Other examples using similar adhesives date back to as recent as 2000 years ago from a well preserved skeleton in the mountains in Norway (*2*). These early adhesives were all inspired and made by using naturally available materials (*1, 2*). Most modern adhesives are synthetically mass produced and are based on chemistries that are not found in natural adhesives. Many of these adhesives are widely successful (e.g., Elmers glue, Super Glue, epoxies). Despite the wide array of adhesives available in the modern world there are still many challenges that have yet to be overcome. One of the largest of which is the ability of adhesives to stick underwater.

Many modern synthetic adhesives are unable to bond in the presence of water. This is mainly due to the inability of most adhesives to displace surface bound waters. In order to form strong bonds the first step is to bind strongly to the surface, forming an adhesive bond. It is this step that fails in most adhesives when in the presence of water (*3*). Often this is due to the chemistry that is at work. The majority of modern adhesives use epoxy/acrylate based chemistry which does not provide the necessary ability to displace water (*4*). Despite this shortcoming of synthetic adhesives, nature has been using adhesives capable of doing this for eons. Specifically, in marine organisms, these unique organisms have, due to their environment, overcome this challenge.

Mussels, barnacles, sea slugs, sandcastle worms, and oysters all are capable of sticking underwater and accomplish this by using specialized adhesive chemistry (*5*). The most well understood of all of these organisms is the marine mussel. After extensive studies many of the mechanisms that marine mussels use to adhere themselves underwater have been elucidated. The marine mussel sticks by placing down a plaque that is connected to soft tissue inside the mussel via a thread as shown in Figure 1.1. This plaque is composed of six different mussel foot proteins (Mfp's) (*6, 7*). While these proteins vary drastically from each other in terms of molecular weight and content the one thing that they have in common is the presence of a unique amino acid 3,4-dihydroxypheylalanine (DOPA) (*6-9*). This amino acid, Figure 1.1, is rarely found in nature and imparts the mussel foot proteins their adhesive properties.

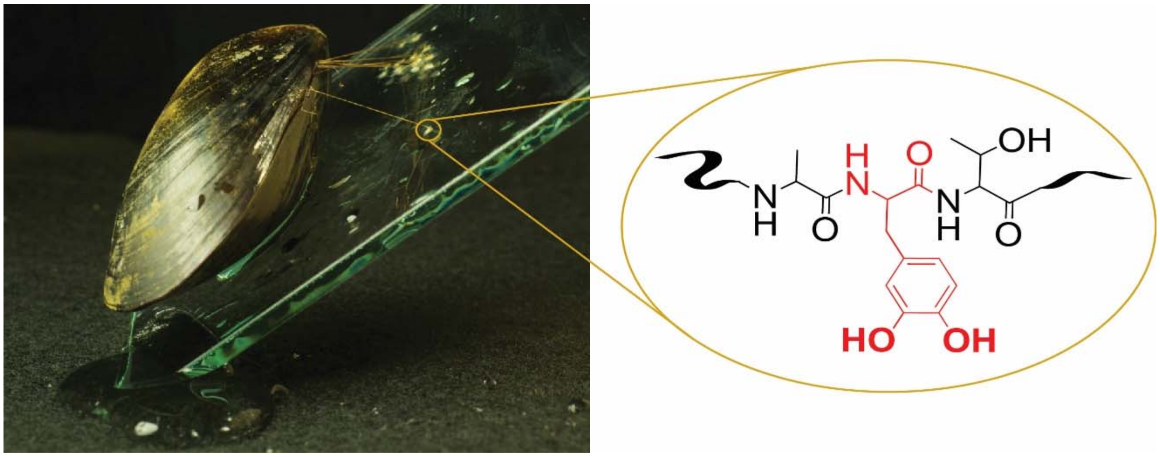

Figure 1.1. Mussel attached to a glass plate using its plaque system. Adhesion is due to the unique amino acid (shown in red) 3,4-dihydroxyphenylalanine.

The proteins contained in the Mfp's each have a different molecular weights and DOPA percentages. Of the six proteins, Mfp-1 is the only protein not involved directly in bonding. Instead, it forms a protective coating around the other five proteins (*6, 7*). Only three of these proteins are in direct contact with the surface that the mussel is trying to bond to. It is these proteins (Mfp's-3, -5, and -6) that provide the surface bonding part of the mussel system (*6, 7*). These three proteins contain the lowest molecular weights of the system, varying from 6,000 - 11,000 g/mol. The DOPA content covers a significant range with Mfp-3 and -5 containing 10 - 20% and 30%, respectively (*6, 7*). Mfp-6 is the outlier of these in that it only has 2% DOPA content. The low molecular weights of these proteins are important because it allows these proteins to wet the surface of the substrates and begin to bind strongly. The other important proteins for adhesion in mussel plaques are Mfp-2 and -4. These two proteins make up the bulk of the mussel plaque and are higher molecular weight than the three in contact with the surface. Mfp-2 and -4 are 45,000 and 90,000 g/mol, respectively, while their DOPA contents are both between 2-5% (*6, 7*). Mussel foot protein 2 and 4 are especially important because, while Mfp-3, -5, and -6 provide surface adhesion, it is the these two that provide the cohesive forces that keep the plaques tethered to the mussels.

These two proteins generate this cohesive force by undergoing cross-linking to form a cured adhesive. The hydroxyl groups of the DOPA (Figure 1.1) are able to undergo chemical cross-linking by different mechanisms, mainly redox reactions, chelation, and radical bonding (Figure 1.2) (*10*). Mussels can utilize iron from their surroundings to chelate in a 3:1 catechol:iron ratio (*10*). Two electron reductions via thiols and amines yield quinones which can covalently bond to metal bound on the surface. Radicals forming from one electron oxidations can bind to other radicals to form covalent bonds between DOPA groups (*10*).

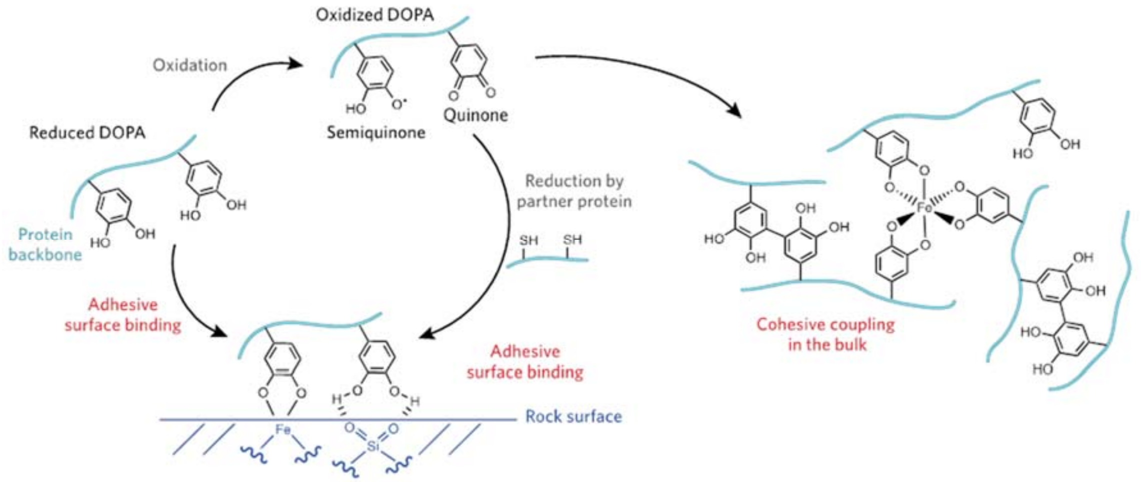

Figure 1.2. Different mechanisms of mussel protein adhesion. The left cycle shows adhesive bonding, the right cohesive. Reprinted with permission from Macmillan Publishers Ltd: *Nature Chemical Biology* (2011) 7, 579-580.

The combination of these cross-linking mechanisms plus the adhesive bonds formed to the surface is what allows the mussel to adhere to a wide array of surfaces underwater, something that many commercial glues are incapable of. This is why many biomimetic systems have used mussels as inspiration for components of their systems.

1.2. Polymer Adhesives: Sticky Solutions

1.2.1. Nature Inspired Materials

Mussel adhesive proteins, while an elegant solution for mussels, are not easily used for other situations. While there are means of forcing mussels to produce glue (*11*) this is still extremely inefficient as the extraction process takes significant time and it is estimated that it would take ~10,000 mussels for 1 gram of protein. This is in addition to the fact that mussels have not evolved to form the strongest adhesive possible. Mussel adhesive proteins connect back to the mussel via a thread that is attached to the internal organs of the mussel. If the adhesive were too strong it would damage the internal organs of the mussel. This in combination with the time and effort required to extract any significant amount of mussel protein make it easier to consider developing a synthetic system that incorporates the desired components of the mussel adhesive protein.

In recent years there has been a blossoming of material systems that mimic various aspects of mussel adhesive proteins (*5, 12-16*). Quite often, synthetic polymers substitute for the protein backbone and derivatives of catechol are appended to these chains for providing the cross-linking and adhesion chemistry of DOPA (Figure 1.1) (*15-27*). Exciting results have been obtained such as, for example, hydrogels being developed with self-healing properties allowing materials to be cut by a knife and then joined back together with recovery of materials properties (*28*). Dry bonding strengths of mussel mimicking polymers have even been able to exceed that of long established commercial products including Super Glue (*27*). While many dry studies have demonstrated the benefits of incorporating mussel adhesive chemistry there are far fewer studies that have demonstrated this in underwater environments.

Making direct comparisons of adhesion data is often difficult, given variations in several parameters including substrate choice, cure conditions, joint type, and testing methods. However, we can gain some context by looking at the strengths prior efforts of underwater adhesive systems are obtaining. Bulk lap shear joints between aluminum substrates with charged catechol containing polymers have been reported at 0.35 MPa for polyoxetanes and humid conditions with partial drying (*22*). When in a coacervate phase of a polyanion condensed with Ca^{2+} cations, strengths up to 1.2 MPa were found (*21*). Neutral catechol-containing polyvinylpyrrolidine applied to wetted glass and then cured underwater was at 1.3 MPa (*24*) and a polyacrylate on wet glass bonded to 1.6 MPa (*29*). Beyond mussel mimicking systems is a "molecular velcro" of a metal complex guest and a macrocycle host, each surface grafted onto silicon, yielding up to 1.1 MPa underwater (*30*). A light cured bispheonol-acrylate adhered aluminum underwater to 1.2 MPa (*31*). There are many ways of incorporating mussel adhesive chemistry but from these prior efforts it can be observed that anything > 2 Mpa would be considered quite strong. Especially when it is taken into account that improvements in bulk adhesive performance are typically gradual.

1.2.2. The Reductionists Approach

In terms of mussel mimicking polymers, the most straightforward one is poly[(3,4-dihydroxystyrene)-*co*-styrene] (Figure 2.1). Catechol groups pendant from a polystyrene host can represent, respectively, the DOPA and polypeptide chains of mussel adhesive proteins (*20, 27*). While the styrene imparts many benefits ranging from being cheap, having no innate adhesive properties, and due to the similarity to 3,4-dihydroxystryene which is easily incorporated into a copolymer. The copolymers can be synthesized on gram scales, thereby enabling bulk adhesion testing. Prior studies have shown that poly[(3,4-dihydroxystyrene)-*co*-styrene] is a quite useful mimic of mussel adhesive proteins in terms of dry bulk bonding performance (*20, 27*). Dry bonding of the polymer is quite appreciable, well into the MPa range for lap shear joints between metal, plastic, and wood substrates (*20, 27*). How well might the dry bonding of this biomimetic system transfer to underwater applications? In terms of polymer composition and molecular weight, which derivatives should bring about the highest underwater bonding performance? Looking at the proteins found in a mussel's adhesive plaque does not provide too much help with regard to design since the DOPA content can range from 3% to 30% of all amino acids (*6*). Molecular weights are as low as 6,000 and as high as 110,000 grams/mole (*7*). Insights into dry bonding with poly[(3,4-dihydroxystyrene)-*co*-styrene] revealed that ~33% mole % 3,4-dihydroxystyrene provides optimal adhesion. This insight, in addition to the effect of other variables (e.g., molecular weight, concentration), has been elucidated by prior studies, in relation to dry bonding, making this simple polymer mimic unique among mussel mimicking adhesives (*18, 20, 23, 27*).

Despite the many adhesives mimicking mussels poly[(3,4-dihydroxystyrene)-*co*-styrene] is one of the few that has undergone systematic studies of dry adhesion. Factors such as fillers, cross-linking, cure conditions, temperature, and time are often considered but only briefly in most mussel mimicking systems. The systematic study of poly[(3,4-

dihydroxystyrene)-*co*-styrene] in addition to the strength, versatility, and availability make it an excellent candidate for further study in an underwater environment.

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CHAPTER 2. ADHESIVE BIOMIMICRY: OUTMUSCLING MARINE MUSSELS

2.1. Introduction

To thoroughly explore the adhesion of polymer systems structure-function studies are needed. For poly[(3,4-dihydroxystyrene)-*co*-styrene] (Figure 2.1), the focus of this study, a large number of these studies have been done previously for dry bonding (*1-4*). The conditions found there, however, do not necessarily carry over to underwater bonding. Underwater bonding carries a significantly different set of challenges then dry bonding. The main problem with underwater adhesions is that many synthetic adhesive fail due to poor substrate adhesion while their cohesive strength is generally quite strong (*5*).

The problems with underwater adhesion can be broken down to two major issues. The first is being able to displace the water molecules present (*6*). The next is the presence of contaminants between adhesive and substrate (*5*). Under dry conditions these issues are either not as significant (i.e., presence of water) or overcome by pretreatment of the surface. Due to the unique adhesion mechanisms of DOPA (e.g., bidentate H-bonds, coordination complexes, and metal chelation) which poly[(3,4-dihydroxystyrene)-*co*-styrene] is able to take advantage of, these issues for underwater adhesion are partially overcome by the materials intrinsic properties.

Previously, our laboratory showed that ~33% mole % 3,4-dihydroxystryene resulted in optimal dry adhesion (*2-4*). While this does not mean it will be optimal for underwater it can serve as a good starting point for an underwater adhesions study. Using this as an initial point various studies were undertaken and the resulting adhesion measured via lap shear and tensile testing. Lap shear and tensile testing as methods to evaluate adhesion are common methods (*7*). Multiple structure-function studies were done to optimize adhesion. The first one was a molecular weight study, followed by a ratio study of % 3,4-dihydroxystryene:% styrene. Once these two were completed additional individual studies of cross-linkers, fillers, cure time, salt content and concentration studies were completed. Once these studies were finished the best adhesive conditions were chosen and adhesion was measured on a wide array of

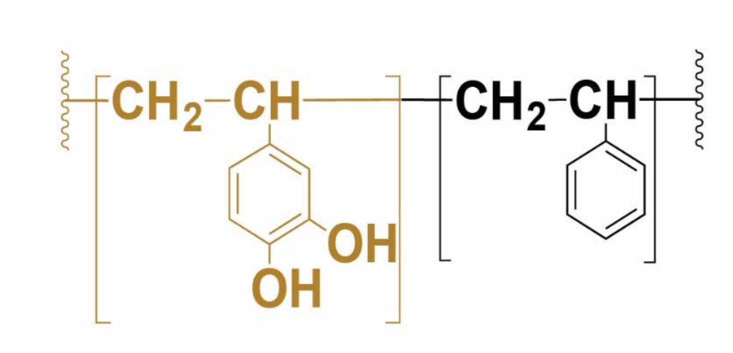

Figure 2.1. Chemical structure of poly[(3,4-dihydroxystyrene)-*co*-styrene] with 3,4-dihydroxystyrene shown in gold and styrene in black.

substrates. The data were then compared to multiple commercial adhesives measured on the same substrates under our testing conditions both in lap shear and tensile. The result of these studies was an underwater adhesive that, under our testing conditions, is equal to or beats all other underwater commercial adhesives tested.

2.2. Adhesive Polymers, a Sticky Synthesis

2.2.1. Synthesis Results

Synthesizing a family of varying poly[(3,4-dihydroxystyrene)-*co*-styrene] polymers was the first step. The general synthesis is well established (*2-4*). The random nature of the synthesis allowed for a spread of different % 3,4-dihydroxystryene incorporations which was further modified by altering the feeds. Additionally, a wide range of molecular weights (M_w = 23,000 – 97,000 g/mol) with low PDI's were able to be synthesized readily by changing the initiator (*n*-BuLi) to monomer ratio added in the beginning of the polymerization. Glass transitions done via DSC demonstrated a single transition temperature of ~ 89 °C, while this exact temperature changes depended on the percent 3,4-dihydroxystryene incorporation and M_w the single transition demonstrates that poly[(3,4-dihydroxystyrene)-*co*-styrene] is a random copolymer. Two transitions would correspond to a block copolymer.

2.2.2. General Synthesis Procedures

The copolymer was synthesized from styrene and 3,4-dimethoxystyrene purchased from Sigma Aldrich. The monomers were purified using an alumina column and an extraction process both methods removed the polymerization inhibitors shipped with the monomers. Polymers were synthesized under argon using Schlenk line techniques using flame dried glassware. Characterization of the poly[(3,4-dimethoxystyrene)-*co*-styrene] composition was done via ^{1}H NMR using a Varian Inova-300 MHz spectrometer (Figure 2.2). Gel permeation chromatography (GPC) with a Polymer Laboratories PL-GPCs20 system provided molecular weights. Glass transition temperatures were provided using a Perking Elmer Jade differential scanning calorimeter (DSC).

Deprotection of poly[(3,4-dimethoxystyrene)-*co*-styrene] was accomplished using Schlenk line techniques under argon using flame dried glassware. Characterization of the deprotected polymer was done via ^{1}H NMR demonstrating the removal of the methoxy peaks. Flame testing of the deprotected polymer was also done to verify the absence of boron in the polymer.

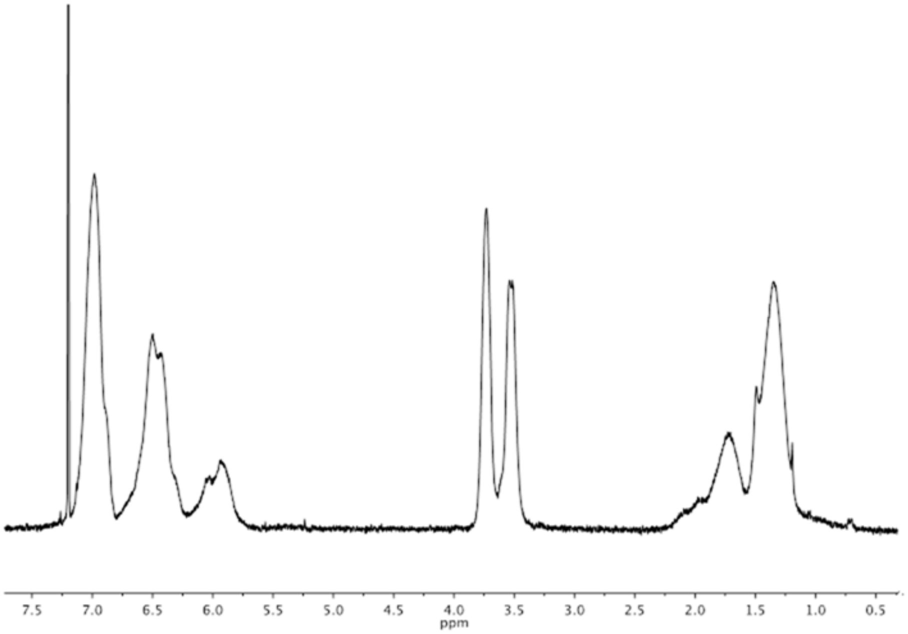

Figure 2.2. Proton NMR of poly[(3,4-dimethoxystyrene)-*co*-styrene]. The removal of the methoxy peaks in the 3.0 – 4.0 ppm range corresponds to successful formation of poly[(3,4-dihydroxystyrene)-*co*-styrene]

2.2.3 Synthesis of poly[(3,4-dihydroxystyrene)-*co*-styrene]

This synthesis is well established (*1-4*). Briefly, styrene and 3,4-dimethoxystyrene are initiated via *n*-BuLi undergoing an anionic polymerization. The resultant poly[(3,4-dimethoxystyrene)-*co*-styrene] is reprecipitated three times with MeOH to crash out the polymer and DCM to dissolve the polymer (*1-4*). After the polymer is dried ^{1}H NMR, GPC, and DSC analysis is done to characterize the polymer. Removal of the methoxy groups is done using boron tribromide. After reacting overnight the mixture is then quenched with MeOH. After which, the polymer solution is washed with 1% HCl and then the supernate decanted. This wash procedure is repeated three times. The resulting white solution is then dissolved in acetone and DCM and dried via rotovap. Removal of the methoxy peaks is confirmed via ^{1}H NMR spectroscopy and residual boron is checked by a flame test with a green flame indicating boron is still present.

2.3. Adhesion Studies

2.3.1. Adhesion Methods

All testing for all the following studies was carried out on an Instron 5544 Materials Testing System at 2 mm/min with a 2,000 N load cell. The majority of the studies were done in shear with single lap-joint configuration on aluminum following a modified version of ASTM D1002 (Figure 2.3) (*8*). The exceptions to this are the substrate studies and the tensile study. Tensile studies were done using the ASTM D2095 standard method (Figure 2.3) (*9*). For all studies five joints was the minimum number of trials collected. All error bars are presented as 90% confidence intervals.

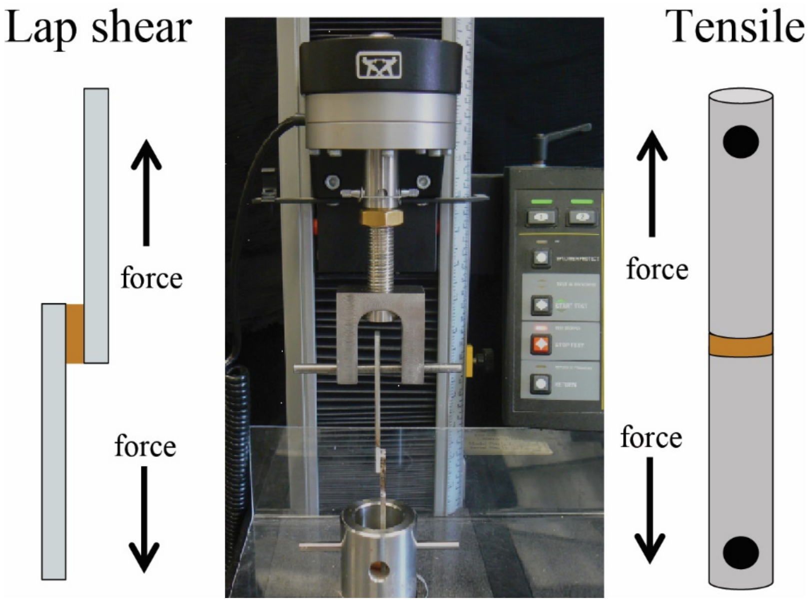

Figure 2.3. Schematics of testing setups for adhesion measurements both lap shear and tensile. Instron Materials testing System loaded with bonded aluminum in lap shear configuration

Aluminum adherends were cut from type 6061 stock and prepared either by mirror polishing with Mibro no. 3 and Mibro no 5. polish or an ASTM D2651-01 method for adherend cleaning (*10*). Red oak was purchased locally and had a surface roughness equivalent to that of sanding with 220 grit sandpaper. Steel adherends were sanded with 50 grit sandpaper prior to testing and then washed with ethanol, acetone, and hexanes. PVC and PTFE were obtained from Rideout Plastics.

Artificial seawater was prepared using Marine Environment dual phase formula and reverse osmosis water to a final salinity of 35 grams/liter. Deionized water was prepared using a Barnstead Nanopure Infinity Ultrapure water system with a final resistivity of 18 MΩ-cm. All water was prepared immediately prior to use. Deionized water was at pH = 8.0 directly from the purifier and at pH = 7.9 after 24 hours. The salt water was pH = 7.9 at both the beginning and end of the 24 hour experiment periods.

Derivatives of poly[(3,4-dihydroxystyrene)-*co*-(styrene)] were dissolved at 0.3 g/ml in chloroform with 45 μL dispensed onto each completely submerged adherend. An additional 15 μL of chloroform was then deposited. Another adherend was placed on top of the first to form overlap joints of 1.2 cm x 1.2 cm. The bonds were cured at room temperature for 24 hours, completely submerged prior to being removed and tested immediately on an Instron 5544 materials testing system. The maximum force at joint failure divided by the overlap area provided the adhesion strength. Each sample was tested a minimum of 5 times and averaged.

All studies were performed based on the results from the prior study, changing only the variable under investigation. After the initial molecular weight study this served as the baseline study from which the others such as the 3,4-dihydroxystyrene, cross-linker, filler, salt, time, and concentration studies were based. Generally, each study was done with the same polymer.

2.3.2. Molecular Weight Influence

Molecular weight can greatly influence adhesion. Prior studies from our lab have demonstrated that increasing molecular weight dramatically enhances adhesion (*2, 3*). Despite this there have been few studies which have done discrete molecular weight optimization with regards to bulk adhesion. Those that do typically consider a narrow range. It only recently that our lab has shown that by considering a broad range of molecular weights a better handle on the effect of molecular weight on adhesion can be grasped.

There are at least two chains of thoughts when it comes to what is a good molecular weight for adhesion. Strong bonds are formed as a result of two modes of bonding i.e. adhesive and cohesive. Adhesive bonding relates to how well the polymer binds to the surface of a substrate while cohesive bonding is the bonding of the polymer to itself in the bulk of the sample. Both of these modes are served by two different molecular weights. Adhesive bonding is generally best with lower molecular weight polymers as they are easier to spread out, penetrate, and provide more contact points onto substrates. Cohesive bonding, on the other hand, is better served by larger molecular weights due to increased chain entanglement which help the polymer resist failure.

For studying the effect of molecular weight on underwater bulk adhesion eleven polymers were synthesized and their adhesion tested (Table 2.1). For this study bulk adhesion for all polymers was tested a minimum of ten times and the catechol content was kept between 27-33%. Similarly to the previous studies molecular weight played a large impact on adhesion. Adhesion increased with polymer molecular weight achieving ~ 2.5 MPa with M_w's ≈ 84,000 g/mol (Figure 2.4). The data show that there is effectively a plateau around ~ 80,000 M_w. Above ~ 90,000 M_w adhesion noticeably declined. This is likely due to the higher molecular weights not penetrating the submerged surface as well as the slightly lower molecular weights. While the lower molecular weights result in less cohesive failure due to fewer bonds in the bulk material.

Table 2.1. Final polymer characterization data for a poly[(3,4-dihydroxystyrene)-*co*-(styrene)] copolymer molecular weight study

final 3,4-dimethoxystyrene content (mole %)	final styrene content (mole %)	M_n (g/mole)	M_w (g/mole)	PDI
31	69	16,000	23,000	1.4
31	69	19,000	30,000	1.6
33	67	29,000	47,000	1.6
27	73	37,000	54,000	1.5
28	72	50,000	67,000	1.3
29	71	49,000	75,000	1.5
29	71	52,000	77,000	1.5
27	73	56,000	79,000	1.4
27	73	59,000	84,000	1.4
28	72	63,000	95,000	1.5
33	67	60,000	97,000	1.6

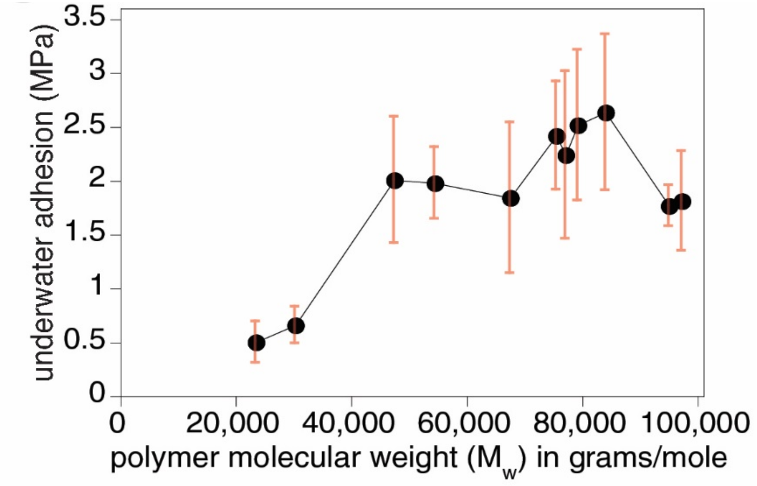

Figure 2.4. Molecular weight effect on adhesion in lap shear of poly[(3,4-dihydroxystyrene)-*co*-(styrene)] copolymer.

2.3.3. Catechol Content

Prior studies (*2-4*) found that dry bulk adhesion was optimized when 3,4-dihydroxystyrene was ~30 mole % 3,4-dihydroxystryene. The percent of 3,4-dihydroxystyrene for optimal adhesion does not necessarily carry over from dry to underwater environments. The presence of water has a large impact on the behavior of the polymer. Despite this these previous data served as a starting point for the above molecular weight study. After finding the ideal molecular weight the percent of 3,4-dihydroxystyrene required for optimal underwater adhesion was revisited.

For studying the effect of 3,4-dihydroxystyrene on adhesion nine different polymers were obtained (Table 2.2). For all of these polymers the molecular weight (M_W) was held within 76,000 – 97,000 giving a ~ 20,000 g/mole range with the sole exception of the 0 % 3,4-dihydroxystyrene point which had a M_W of 101,000 and was purchased from Sigma Aldrich. Similarly to the molecular weight study each point was tested a minimum of ten times. The 3,4-dihydroxystyrene content ranged from 0 - 36 %. The varied incorporations displayed a significant effect on adhesion (Figure 2.5).

The lower mole % 3,4-dihydroxystyrene polymers displayed little to no adhesion. At 22 % 3,4-dihydroxystyrene was where adhesion peaked. After the peak adhesion decreased a little but plateaued and was still significant. Above 30 % 3,4-dihydroxystyrene adhesion was significantly decreased from the peak but still respectable at ~ 1.5 MPa (Figure 2.5). Compared to the optimal dry adhesion composition of ~ 33 % 3,4-dihydroxystyrene underwater is significantly lower than the ideal composition. The reason for this is presence of water and different substrate interactions. Lower 3,4-dihydroxystyrene content might be ideal for displacing water and good cohesive bonding while higher contents might be causing the polymer to favor cohesive bonding over strong bonds to the surface.

Table 2.2. Final poly[(3,4-dihydroxystyrene)-*co*-(styrene)] polymers used for a composition study

final 3,4-dimethoxystyrene content (mole %)	final styrene content (mole %)	M_n (g/mole)	M_w (g/mole)	PDI
0	100	97,000	101,000	1.0
17	83	67,000	96,000	1.4
21	79	63,000	84,000	1.3
24	76	67,000	97,000	1.4
27	73	59,000	84,000	1.4
29	71	52,000	77,000	1.5
30	70	49,000	75,000	1.5
33	67	60,000	97,000	1.6
36	64	53,000	76,000	1.4

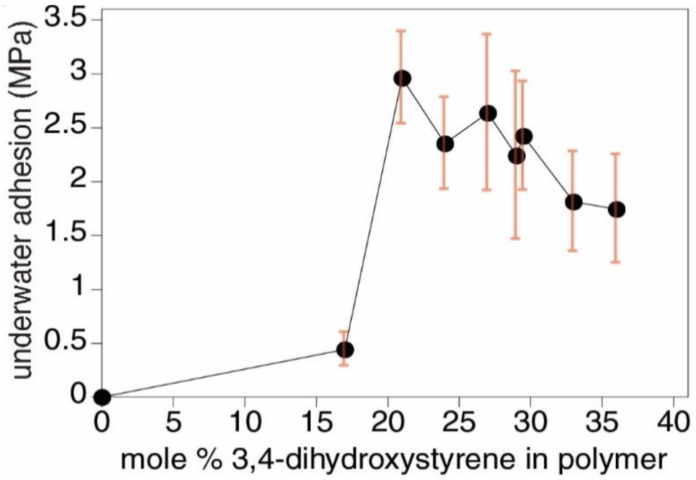

Figure 2.5. Varied 3,4-dihydroxystyrene effect on adhesion in lap shear of the poly[(3,4-dihydroxystyrene)-*co*-(styrene)] copolymer

2.3.4. Cross-linking

Many commercial adhesives use cross-linking to enhance adhesion. This same strategy for increasing adhesion is found in marine mussels (*11-13*). Cross-linking is accomplished by a number of different mechanisms. Typically it involves manipulating redox chemistry to form covalent bonds in the bulk material. Other mechanisms are metal coordination, enzyme mechanisms, and hydrogen bonding (*14-17*). Many studies on new systems use cross-linking chemistry including the prior studies on poly[(3,4-dihydroxystyrene)-*co*-(styrene)] (*4*). After testing a wide array of cross-linking agents it was found that dry adhesion was significantly increased when tetrabutylammonium periodate was added in a 1:3 periodate to catechol ratio. This cross-linking enhanced both the cohesive and adhesive bonding of the dry system.

Similar enhancements were investigated for applicability to underwater applications with the prior results serving as a starting point. The first ratio that was tested was the same 1:3 periodate to 3,4-dihydroxystyrene ratio that had demonstrated the best adhesion increase. Quick studies with this revealed that cross-linking was happening too rapidly to allow for formation of the lap shear bond. Using this information the ratio was then adjusted to lower ratios. Three cross-linker to catechol ratios were investigated, 1:10, 1:50, and 1:100. Each cross-linker was tested a minimum of five times at each of the three different ratios.

Many different cross-linkers were selected for cross-linking of the polymer system underwater. These cross-linkers had been used previously for dry adhesion studies (*4*). Some of these cross-linking agents mimic chemistries that are found in the mussel. Mussels contain high concentrations of Fe, Mn, Cu, and Zn all of which are extracted from their surroundings. Eight different cross-linking agents were explored ranging from metal containing to nonmetallic cross-linkers (Figure 2.6).

The cross-linkers that contained metal were iron(III) nitrate ($Fe^{III}(NO_3)_3$), iron(III) acetonylacetonate ($Fe^{III}(acac)_3$), tetrabutylammonium dichromate ($[(C_4H_9)_4N]_2Cr^{VI}{}_2O_7]$), and potassium ferrate ($K_2Fe^{VI}O_4$). The nonmetallic cross-linkers tested were tetrabutylammonium periodate ($[(C_4H_9)_4N]IO_4$), *tert*-butyl peroxide *t*-BuOOH, cumene hydroperoxide ($C_6H_5C(CH_3)_2OOH$), and methyl ethyl ketone peroxide ($(H_3CCH_2COCH_2O)_2$).

Cross-linking at 1:10 cross-linker to catechol ratio across all eight cross-linking agents displayed no increase in adhesion (Figure 2.6-A). A large decrease in adhesion was noted for all cross-linkers except for the two hydroperoxides. This indicates that the cross-linking concentration was too high, as the system preferred cohesive bonding vs. adhesive, even at ratios over three times greater than that of the one found for dry adhesion. Diluting the concentration of the cross-linker even further to a 1:50 ratio revealed increased adhesion across all cross-linkers (Figure 2.6-B). Despite this the data still showed that cross-linking provides no benefit over the polymer alone system. Taking the dilution one step further, to a ratio of 1:100 cross-linker to catechol, revealed increased adhesion for most of the cross-linkers (Figure 2.6-C). This time the peroxides uniformly demonstrated higher average adhesion values. But when 90% confidence intervals were taken into account they were not significantly different from the polymer alone adhesion. Taking this into consideration all further studies where done without the presence of cross-linking agents.

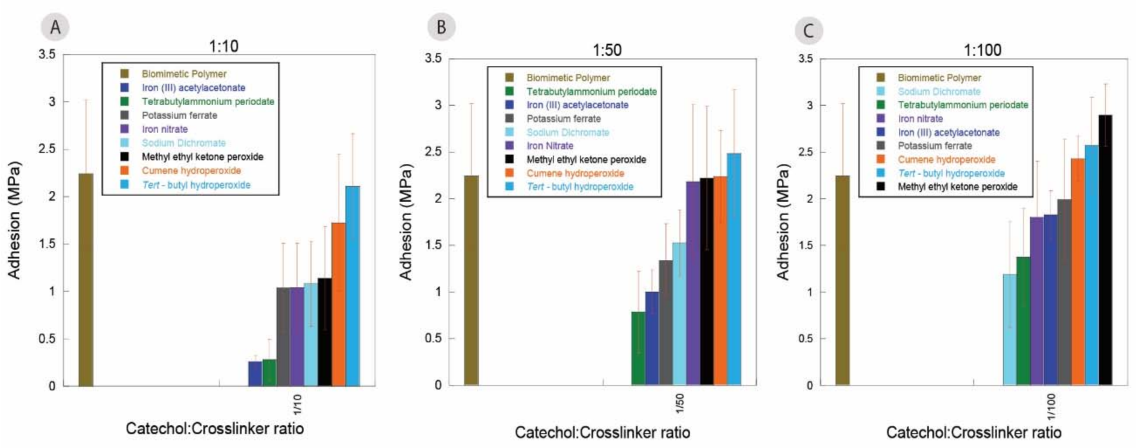

Figure 2.6. Effect of a variety of cross-linkers on poly[(3,4-dihydroxystyrene)-*co*-(styrene)] copolymer at three different ratios.

2.3.5. Fillers

In addition to cross-linking another common way of enhancing polymers is the use of fillers to form a composite (*18, 19*). Fillers serve as a reinforcing material imparting beneficial properties such as higher strength, stiffness or flexibility (*20*). The presence of fillers however, has significant considerations ranging from polymer filler interactions, filler properties (i.e. size, shape, chemical properties), and the amount of filler present (*21*). Fillers are capable of great enhancements yet due to all the variables care must be taken to ensure that they are not a detriment.

The most common fillers used for adhesives are glass fibers, cellulose fibers, and calcium carbonate. It is these three that were thoroughly investigated here (*4*). Others such as carbon fibers, laponite, and fumed silica were also briefly examined. Prior results with calcium carbonate showed that optimal adhesion was obtained with 3.5 µm $CaCO_3$ so the same size was used for this study. Shorter fiber lengths, less than a mm, have been shown in previous systems to provide adhesive gains so these lengths were chosen for this study (*4*). All fillers were dispensed into the polymer solution via vortexing.

Due to the differences even a slight difference in filler content can have, a range of fillers were studied for three common fillers in wide spread commercial use. For the other fillers the best percentage that had been found for the three commercial fillers was tested. Standard fillers comprised 10 – 50% by weight of the solution. For these studies a range of 2.5 – 30 % was studied. The higher percentages where attempted but not successful due to poor solubility at higher concentrations.

All three commercial fillers where done with the same polymer and tested a minimum of five times. Adhesion differed greatly between the commercial fillers (Figure 2.7). For the cellulose fibers adhesion was uniformly lower than the polymer alone system no matter the weight content. For the $CaCO_3$ only the 10% filler content displayed an average adhesion that was greater than polymer alone. Yet when 90% confidence intervals are considered adhesion is not statistically different. The glass fibers displayed the most uniform results. All weight percent’s over 5 % glass fibers had a higher average adhesion than polymer alone, however, they were not significantly different from the polymer alone system.

Other filler included fumed silica, both hydrophilic & hydrophobic, carbon fibers and laponite. The fumed silica was tested at 10 % by weight and provided no benefit to adhesion, like the glass fibers. The carbon fibers where not soluble in the standard polymer solution. Mean while the laponite also resulted in problems with compatibility with the system. Taking this into consideration all further studies where done without the presence of fillers.

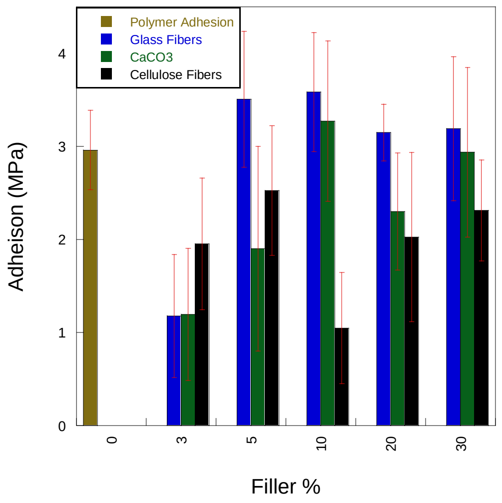

Figure 2.7. The effect of different filler weight percents on adhesion with poly[(3,4-dihydroxystyrene)-*co*-(styrene)].

2.3.6. Salty Times

From these studies we have shown that adhesion with poly[(3,4-dihydroxystyrene)-*co*-(styrene)] can be quite significant yet enhancement of this adhesion via cross-linking and fillers has proven to be minimal at best. These two methodologies are the most common ways found in nature and manmade materials for affecting adhesion under any environment. There are other considerations that must be taken into account when underwater adhesion is considered by itself, specifically, the role of charge and salt.

Cationic charges within mussel adhesive proteins have been proposed recently to aid bonding in salt water (*22*). Positively charged amino acids could help outcompete surface-bound cations such as sodium, thereby allowing proteins to gain access onto mildly anionic surfaces including rocks (*22*). In order to address potential roles for charges (*1, 22*) and salts (*1, 22-25*), we examined the bonding of poly[(3,4-dhydroxystyrene)$_{28\%}$-co-(styrene)$_{72\%}$] (M_w = 95,000 grams/mole) in deionized water (pH = 7.9) and found a value of 0.4 ± 0.1 MPa. When the same experiment was carried out under artificial sea water (pH = 7.9), adhesion was at 1.8 ± 0.2 MPa. Note that poly[(3,4-dihydroxystyrene)-*co*-(styrene)] is a neutral polymer and we might expect improved adhesion under deionized versus salt water. This unexpected finding could be a function of the current study using a bulk, macroscopic adhesion method versus prior efforts examining interactions on the nanometer scale (*22*). Nonetheless, such data indicate that disruption of charges atop submerged surfaces may not be of primary importance for adhesion in the seas.

2.3.7. Synthetic Comparisons

Making direct comparisons of adhesion data is often difficult, given variations in several parameters including substrate choice, cure conditions, joint type, and testing methods. In order to provide benchmarking of poly[(3,4-dihydroxystyrene)-*co*-(styrene)] adhesives strength compared to commercial adhesives bonding was carried out with a range of commercial glues including common adhesives and specialty materials billed for wet applications. Conditions held constant included quantity of adhesive, cure time, cure temperature, substrate, and being applied underwater. Figure 2.8 provides data showing that poly[(3,4-dihydroxystyrene)-*co*-(styrene)] outperformed every product tested, usually by quite significant margins. Standard adhesives such as Elmer's Glue-All (polyvinyl acetate) and Super Glue (ethylcyanoacrylate) failed to bond at even modest levels, likely a result of not being able to cure underwater or the water inducing curing too rapidly to allow interaction with the substrates, respectively.

For providing a broader context of underwater bonding capability, poly[(3,4-dihydroxystyrene)-*co*-(styrene)] adhesion on a range of different substrates was compared to the five strongest underwater commercial glues from Figure 2.8. Table 2.3 shows that these selected commercial products performed best with polyvinyl chloride, etched aluminum, and sanded steel substrates. On polytetrafluoroethylene (Teflon), wood (red oak), and polished aluminum, catechol-polystyrene displayed the highest adhesion. With Teflon, only the biomimetic polymer and a single product provided any bonding at all. For wood, catechol-polystyrene was the single system capable of creating a measurable bond underwater.

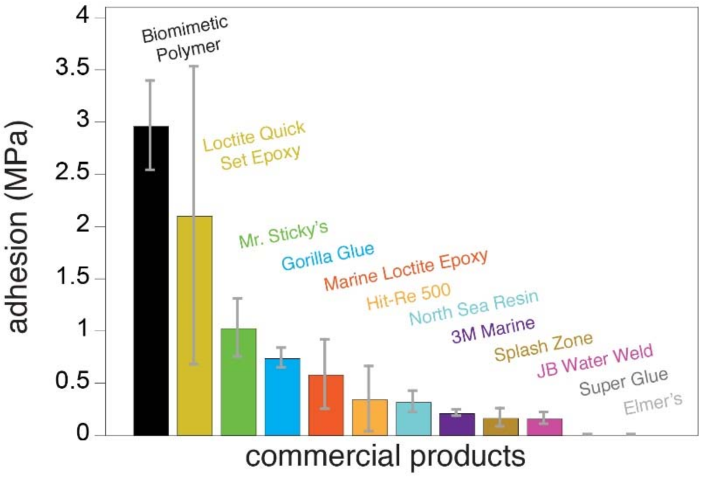

Figure 2.8. Comparison of commercial products to poly[(3,4-dihydroxystyrene)-*co*-(styrene)] in lap shear on polished aluminum.

Table 2.3. Comparison of substrates and commercial glues to poly[(3,4-dihydroxystyrene)-*co*-(styrene)] in lap shear

adhesive	substrate					
	polished aluminum	etched aluminum	sanded steel	Wood	PVC	Teflon
Mr. Sticky's	1.0 ± 0.3	0.2 ± 0.1	0.4 ± 0.1	0	3.0 ± 0.6	0.1 ± 0.1
Marine Loctite epoxy	0.6 ± 0.3	0	0.2 ± 0.1	0	2.0 ± 0.5	0
3M Marine sealant	0.20 ± 0.03	0.10 ± 0.02	0.2 ± 0.1	0	1.0 ± 0.3	0
North Sea Resin	0.3 ± 0.1	0	0.2 ± 0.1	0	0	0
Gorilla Glue	0.7 ± 0.1	0.4 ± 0.2	0.5 ± 0.1	0	3.0 ± 0.6	0
Biomimetic Polymer	3.0 ± 0.4	0.2 ± 0.1	0.10 ± 0.02	0.20 ± 0.02	0.4 ± 0.1	0.3 ± 0.1

2.3.8. Natural Comparisons

A new material that can, at times, outperform established products is quite exciting (*5, 26-28*). Perhaps even more challenging is direct comparison of a biomimetic system against the true biological counterpart. Very few bio inspired materials are capable of duplicating the full extent of the system that begets them. For poly[(3,4-dihydroxystyrene)-*co*-(styrene)] the comparison to live mussels required changing the testing methods from lap shear to tensile.

Live mussels were placed atop sheets of aluminum, polished by the same method as for lap shear testing, and allowed to deposit there adhesive. Using an established method and several animals, adhesive plaques were pulled up from the surfaces until failure (*29*). Average mussel adhesion in this tensile mode was 0.13 ± 0.01 MPa. For a direct comparison, polished aluminum rods were held under salt water and bonded together into tensile joints using catechol-polystyrene. The rods were then pulled apart to reveal adhesion at 2.2 ± 0.9 MPa. This is over a magnitude difference in adhesion strength. One possibility for this difference is that our efforts have been aimed at creating the strongest underwater glue possible. A living mussel, by contrast, need only attach strongly enough to deter the forces exerted by waves and predators. Perhaps such differences in end goals can, at least partially, explain how catechol-polystyrene outperformed mussel adhesive.

In order to provide a broader context of the mussel plaque to poly[(3,4-dihydroxystyrene)-*co*-(styrene)] comparison the same commercial glues that were tested and compared via lap shear in the prior section were repeated in tensile (Figure 2.9). This testing was done under similar conditions of the mussel testing. A minimum of five tests where done for each point and the same variables were controlled. This time, however, since the mussel plaques testing procedure calls for three days, curing the tensile tests were also allowed to cure for three days before testing.

As shown in Figure 2.8 poly[(3,4-dihydroxystyrene)-*co*-(styrene)] outperforms the majority of all other commercial adhesives in lap shear. In tensile, however, unlike shear there are two others that are on par with poly[(3,4-dihydroxystyrene)-*co*-(styrene)] Loctite quick set epoxy and Marine Loctite epoxy. This increase in bonding is likely due to the increased cure time of three days. This also shows that a majority of commercial systems are capable of outbonding the native marine mussel but all of the other commercial products use synthetic chemistry to adhere. Given that the commercial glues have been around for up to decades, we are excited to report excellent performance for a relatively young biomimetic system.

Being able to outperform the reference biological system in terms of adhesion is impressive but the marine mussel is able to stick not only in many environments, as is poly[(3,4-dihydroxystyrene)-*co*-(styrene)], but is also capable of forming a lasting bond. Marine mussels are able to bind in place and remain there until they wish to detach. This property is difficult to replicate as water seeps into bond lines and degrades the bond (*6, 30, 31*). In order to gauge how resistant poly[(3,4-dihydroxystyrene)-*co*-(styrene)] is to water influx a time study was carried out.

To provide comparison to the marine mussel all testing was carried out in salt water which was held at the same salinity and pH as saltwater throughout the entire testing time. Each point is a minimum of five trials and was set up in a lap shear configuration. Figure 2.10 shows that poly[(3,4-dihydroxystyrene)-*co*-(styrene)] is capable of forming a lasting bond. The adhesion strength peaks after three days and plateaus for one week until finally it decreased after two weeks. Despite this decrease in strength the adhesion remains quite high even after three months have passed.

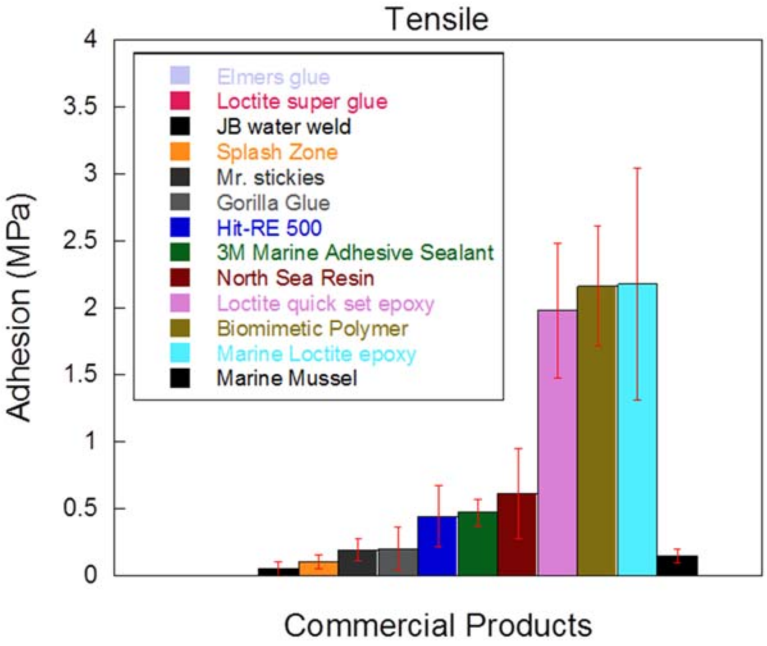

Figure 2.9. Comparison of commercial products and poly[(3,4-dihydroxystyrene)-*co*-(styrene)] to the marine mussel.

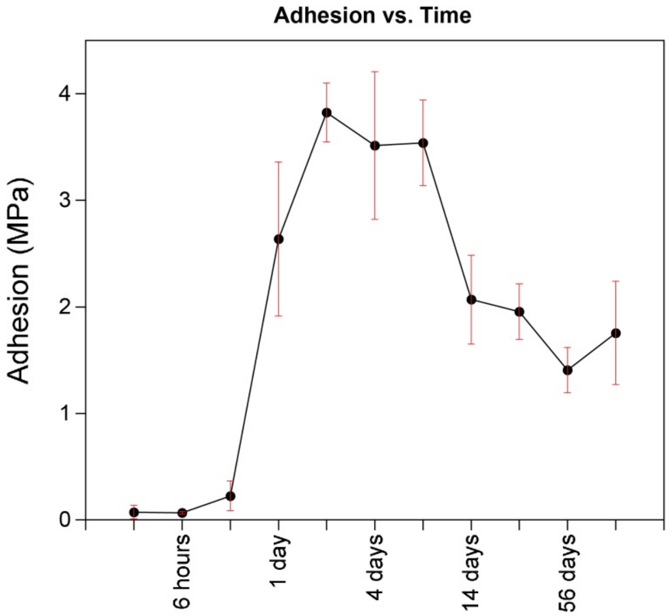

Figure 2.10. Adhesion vs. time cured in salt water and adhesion measured via lap shear

2.4. Concluding Remarks

Results presented here show that man-made materials can, indeed, bring about quite significant underwater adhesion. Biomimetic principles are useful for developing materials that outperform commercial products and even, surprisingly, a reference biological system. By performing systematic structure-property studies it is revealed that the ideal formulation for poly[(3,4-dihydroxystyrene)-*co*-(styrene)] with regards to underwater adhesion is polymer alone without any of the tested additives. While there were some benefits both with cross-linkers and fillers these where not significantly different and when combined together into a consensus study actually resulted in lowered adhesion.

Efforts also demonstrated that poly[(3,4-dihydroxystyrene)-*co*-(styrene)] is capable of outperforming the majority of commercial products available both in lap shear and tensile under certain conditions. Not only that but the bond from poly[(3,4-dihydroxystyrene)-*co*-(styrene)] is long lasting, being able to retain significant adhesion out to three months. All of these data demonstrate the versatility of poly[(3,4-dihydroxystyrene)-*co*-(styrene)] in being able to not only have significant dry adhesion, as demonstrated previously, but also be one of the strongest underwater adhesives tested to date.

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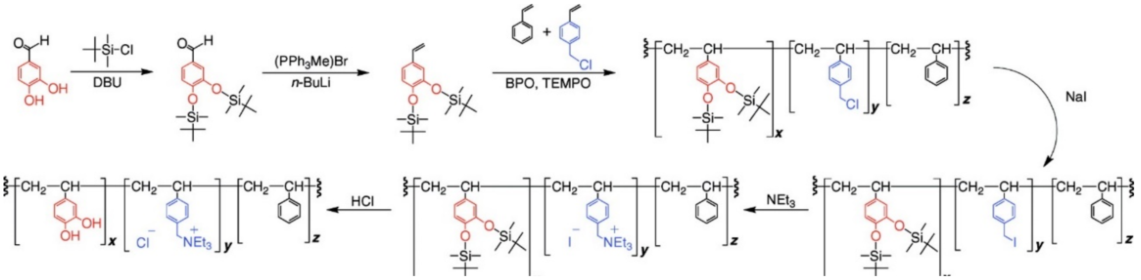
CHAPTER 3. REDESIGNING A BIOMIMETIC POLYMER: A CHARGED ISSUE

3.1. Introduction

Reducing mussel adhesive proteins to a simple copolymer system yielded outstanding results both in dry and wet adhesion (*1-4*). Having explored a variety of variables and achieving such high bond strengths the question then becomes how can it be further improved? The first place to look for inspiration is back at the marine mussel proteins. The marine mussel takes advantage of charged amino acids to promote strong surface binding but despite this it is still unknown how important the charged amino acids are for strong surface bonding.

Several recent reports have been contributing to our understanding of how mussels bond so well. Catechols have a special ability to drill down through surface-bound waters for enabling wet attachment. Having two, adjacent alcohol groups might allow for cooperative binding, analogous to an entropic "chelate effect" (*5, 6*). Hydrogen bonding and metal chelation at the substrate appear likely to be contributing surface adhesion (*5*). Oxidative cross-linking generates cohesive forces (*7-9*). Cationic charges within mussel adhesive proteins have been proposed recently to aid this bonding in salt water (*10*). Positively charged amino acids could help outcompete surface-bound cations such as sodium, thereby allowing proteins to gain access onto mildly anionic surfaces including rocks (*10*). The role of salt was addressed in a previous chapter where it was revealed that bonding in salt water results in significantly higher adhesion then in deionized water. Such data indicated that disruption of charges atop submerged surfaces may not be of primary importance for adhesion in the seas. However, in order to address the potential for charge to impact underwater adhesion a polymer incorporating charge was targeted by synthetic efforts.

This is not the first time that charge-containing polymers have been targeted by our group. Previously, positively and negatively charged polymers have been made and tested. These polymers displayed adhesion rivaling that of the neutral polymers plus better wetting properties (*1*). Despite this apparent success these polymers proved to have a very large synthetic cost (*1*). As seen in Scheme 3.1 the first steps involved the protection of the hydroxyl groups and formation of the terminal alkene group via the Wittig reaction (*1*). After the monomer formation, it was then polymerized forming the general backbone of the polymer (*1*). In order to insert the positive charge the polymer underwent a halide exchange, from chloride to iodide, allowing triethylamine to replace the halide group easier at this point. Then the protecting groups were removed via acid. Being six steps and having an overall yield of ~ 20% resulted in a significant expenditure of time in solely producing the polymer (Scheme 3.1) (*1*). The rest of this chapter details synthetic effort to form similarly charged polymers but using fewer steps, providing better yields all aimed at lowering the synthetic cost of forming these polymers.



Scheme 3.1. The chemical scheme for synthesis of cationic polymer via previous synthetic methods. Reprinted with permission from American Chemical Society *Macromolecules* (2011) 44 (13), pp 5085–5088

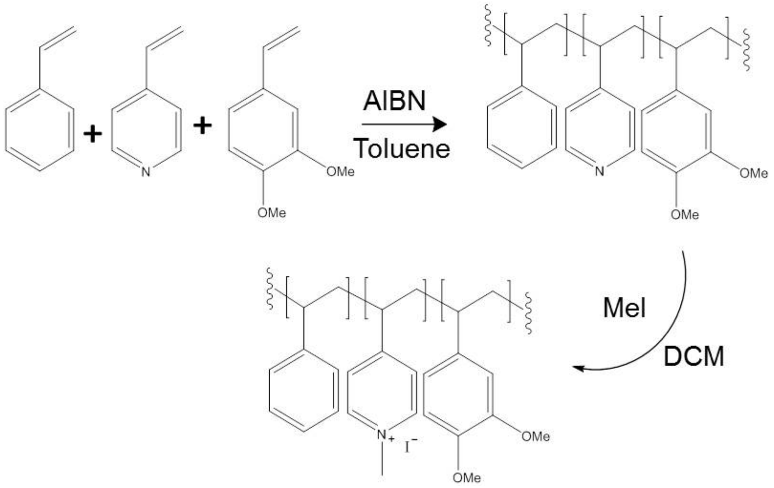
3.2. Amine Problem

The first alternate route to a charged polymer that was explored was using 4-vinyl pyridine polymerized with 3,4-dimethoxystyrene and styrene. The pyridine ring maintains the same structure as that found in poly[(3,4-dihydroxystyrene)-*co*-(styrene)] but adds another functional group in the amine that allows for charge incorporation. This monomer due to its similarity is susceptible to the same types of initiation and purification as the styrene and 3,4-dimethoxystyrene monomers.

A range of copolymers incorporating 4-vinylpyridine with styrene or 3,4-dimethoxystyrene were made in addition to an array of terpolymers of 4-vinyl pyridine styrene, and 3,4-dimethoxystyrene (Scheme 3.2). The general synthesis was modeled after the one used previously for poly[(3,4-dihydroxystyrene)-*co*-(styrene)]. The only fundamental difference was that instead of the anionic initiator *n*-BuLi a radical initiator (AIBN) was used. By varying the feed ratios and the weight percent AIBN a spread of different polymers was obtained. After formation of the polymers the nitrogen on the pyridine was methylated using methyl iodide to yield a charged polymer. At this stage, however, the removal of the methoxy groups from the 3,4-dimethoxystyrene proved problematic (Scheme 3.2).

The standard practice in our lab for the removal of methoxy protecting groups is using BBr_3. When this was attempted on poly[(N-methyl-4-vinylpyridinium)-*co*-(3,4-dimethoxytyrene)] and poly[(N-methyl-4-vinylpyridinium)-*co*-(3,4-dimethoxystyrene)-*co*-(styrene)] it failed to remove the methyl groups of the 3,4-dimethoxystyrene. This is likely due to the positive charge interfering with the mechanism of the BBr_3 deprotection which utilizes charge in ether cleavage (*11*). Methylation was attempted after the deprotection but proved to not be feasible because it did not selectively add the methyl groups back to the nitrogen and methylated the free hydroxyl groups.

Alternate routes of removing the methyl groups were attempted including deprotection reagents such as TMSI (*12*), NASEt (*13*), and Pyr·HCl (*12*). All of these reagents were used both on poly[(N-methyl-4-vinylpyridinium)-*co*-(3,4-dimethoxytyrene)] and poly[(N-methyl-4-vinylpyridinium)-*co*-(3,4-dimethoxytyrene)-*co*-(styrene)]. None of them were successful at removing the methyl groups selectively from the aryl ether. This is again most likely due to the presence of the positive charge in the polymer interfering with the mechanism.

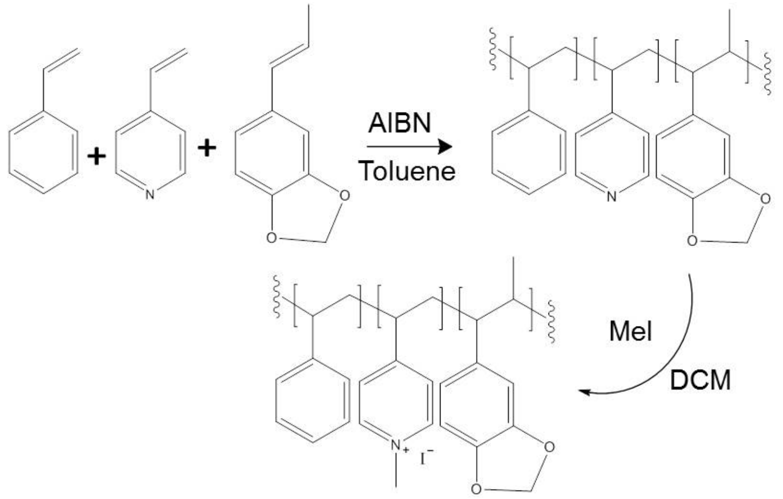


Scheme 3.2. Terpolymer synthesis and methylation with vinyl pyridine

3.2.1. Bridging the Problem

Despite the successful formation of a cationic charged polymer with so many issues stemming from the removal of the aryl methyl ethers alternative protecting groups were investigated. Another popular protecting group for catecholic chemistry is using a methylene bridge between the meta and para positions. This is easily purchased in the form of isosafrole. Isosafrole is an organic compound derived from safrole. Isosafrole was polymerized using AIBN with 4-vinylpyridine to form the copolymers as well as terpolymers also incorporating styrene (Scheme 3.3).

The deprotection strategies for the bridging methylene group used the same reagents as the aryl methyl ethers. Namely BBr_3, TMSI, NaSEt, and Pyr·HCl. These reagents which were previously ineffective when there where two sites to deprotect. Here we hoped to be more effective on a single group that was protecting both oxygens. This proved to not be case as none of the reagents used for the removal of methylene bridges worked for poly[(N-methyl-4-vinylpyridinium)-*co*-(3,4-dimethoxystyrene)] or poly[(N-methyl-4-vinylpyridinium)-*co*-(3,4-dimethoxystyrene)-*co*-(styrene)]. After the inability to remove the methylene bridge was discovered the protecting group was changed to a different group more reminiscent of the previous six step synthesis.



Scheme 3.3. Terpolymer synthesis and methylation with Isosafrole.

3.3. Silylation

The original synthetic scheme (Scheme 3.1) for the cationically charged polymer made use of silicon protecting groups, in the form of *tert*-butyldimethylsilyl groups, to form an aryl silyl ether complex (*1*). These groups were present throughout the entire synthesis until the last step where they were removed with acid (*1*). The susceptibility of the silyl groups to removal via acid made them a prime target for use as a protecting group in a new synthetic scheme. The charged nature of the polymer does not interfere with the removal of the silyl groups as acid is much less susceptible to interference via charge.

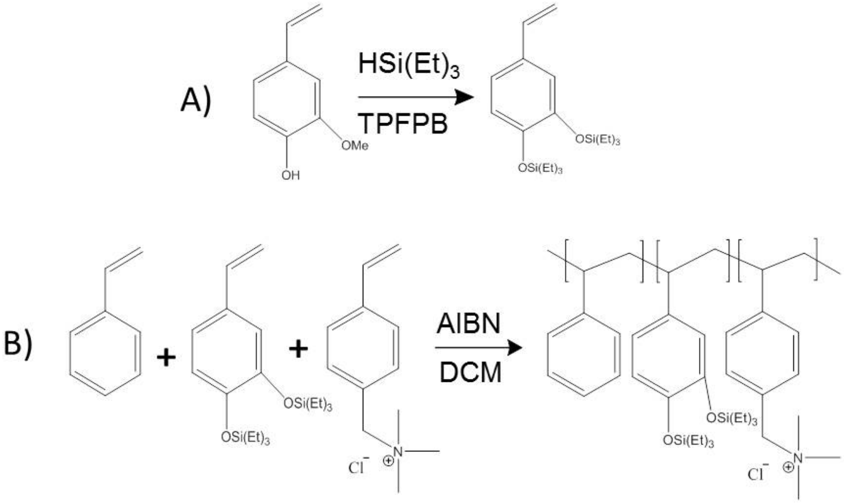
Before this advantageous chemistry could be manipulated the silyl groups must first be added. In the initial incarnation the aryl silyl ethers were added via a straight forward silylation followed by a Wittig reaction to form the monomer (*1*). However, recent literature provided a means to add them more directly (*14*). Using 2-methoxy-4-vinylphenol in the presence of a catalyst (tris(pentaflurophenyl)borane) allowed for the silylated monomer to be formed directly by passing the need for the Wittig reaction (*1, 14*). Further shortening of this synthesis was found to be possible in the form of vinylbenzyl trimethylammonium chloride. Due to the already charged nature of this monomer it allows two additional steps to be removed from the original synthesis. This brings the hypothetical number of steps needed down to three, the formation of the silylated monomer, polymerization and deprotection.

3.3.1. Synthesis

The synthesis of positively charged polymers begins with first silylating 2-methoxy-4-vinylphenol. This is done according to the literature methods (*14*). Briefly, this is done by mixing 2-methoxy-4-vinylphenol with triethylsilane and letting them mix for five minutes in air. After mixing then the catalyst, tris-(pentaflurophenyl)borane, can be added slowly. Addition of too much catalyst causes a dark red color, rapid formation of gas and bubbling of the solution. The solution is then mixed for 10 minutes at room temperature in air. After which it is run through a neutral alumina column with DCM if necessary. After being run through the column the triethylsilane protected monomer is concentrated via a rotovap (Scheme 3.4-A).

The polymerization is run under standard Schlenk line conditions with a flame dried flask. In a standard reaction styrene (14.8 mmol), vinylbenzyl trimethylammonium chloride (10.3 mmol), and the silylated monomer (8.13 mmol) are combined in DCM inside the flame dried flask with the free radical initiator AIBN (0.29 mmol). These are allowed to react at 70 degrees Celsius overnight. These ratios are subject to change depending on what the target polymer is. The reaction then is worked up by sonicating in ethanol and then reprecipitating in ether. This is repeated three times. This affords a protected charged polymer in a straight forward manner in only two steps (Scheme 3.4-B).

This polymer is confirmed via ^{1}H NMR in DMSO (Figure 3.1). The silyl protecting groups are observed at ~0.95 ppm and 0.51 ppm. Their presence is in agreement with literature values of their location in similar polymers and ensured that the catechol groups would be protected (*14*). These peaks will also serve as indicators of how well any deprotection attempts are as ideally those peaks will disappear and be replaced with an –OH peak as shown in Figure 3.2.



Scheme 3.4. Two step synthesis of a cationic charged polymer.

3.3.2. Deprotection

The triethylsilane protecting groups allow for a different mechanism of removal then previous protection strategies. In the initial cationic polymer the silyl protecting groups were removed with the strong acid HCl. In this system HCl was enough to allow complete removal of the protecting groups. Using this information the first acid that was investigated for the new cationic charged polymer was HCl.

Unlike the previous system that used *tert*-butyldimethylsilyl groups which were easily removed with HCl the triethylsilyl groups do not get fully removed in the presence of concentrated HCl. While they may not be fully removed after being subjected to concentrated acid this does provide indication that the right acid may be capable of removing these groups as a peak corresponding to an –OH is observed.

After this success with concentrated HCl others acids were looked at for the deprotection. The first acid that was investigated was TFA. This acid is used often for deprotecting silyl groups and has been used by our lab previously for deprotecting *tert*-butyldimethylsilyl and *tert*-butyldiphenylsilyl while forming a peptide (*15*). Deprotection with TFA was attempted by dissolving the charged terpolymer in EtOH with excess TFA and allowed to stir for 24 hours. This yielded the ^{1}H NMR shown in Figure 3.2. There is a noticeable decline in the ^{1}H NMR signals corresponding to the silyl protecting groups but they have not vanished completely. This is not entirely unexpected as prior results with TFA showed that it was only capable of deprotecting ~ 90% of the silyl groups (*15*).

Previously this was overcome by incorporating a source of fluorine in the deprotection reaction (*15*). This takes advantage of the strong bond between Si and F to help drive the deprotection to completion. Prior efforts in our lab made use of this by using TBAF to provide the fluorine (*15*). In the new charged terpolymer system TBAF was tried at first but was not soluble in similar solvents to the polymer and thus was not able drive it any closer to full deprotection. An alternative source of fluorine was found in the form of NH_4F. There have been other groups that have used this to drive the deprotection reaction to completion and was soluble in the same solvent as the polymer.

When used in the literature, NH_4F is used by itself without acid present, initial experiments where done mimicking these conditions. It was found that NH_4F by itself was not enough to deprotect the cationic terpolymer. Building off the labs previous work where it took a combination of both the strong acid TFA and TBAF to fully remove the silyl protecting groups similar analogous conditions are being explored for the cationic terpolymer (*15*).

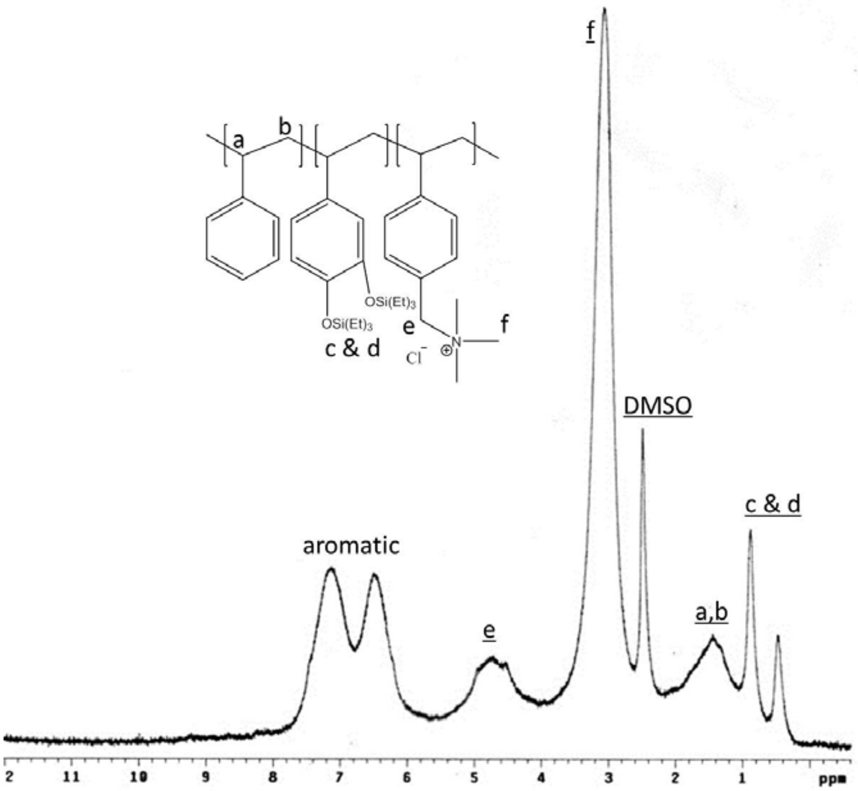


Figure 3.1. ^{1}H NMR of cationic terpolymer protected with triethylsilyl groups.

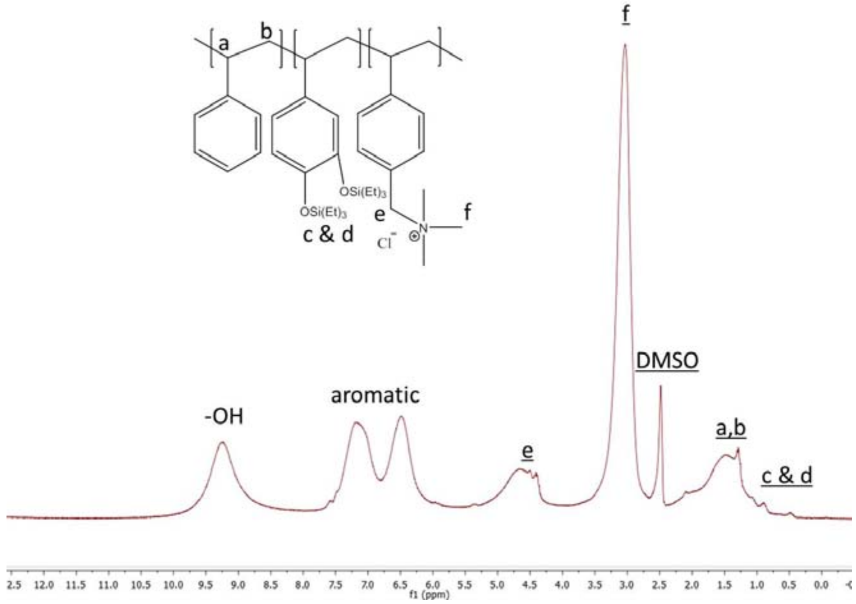


Figure 3.2. ^{1}H NMR of cationic terpolymer with a majority of the triethylsilyl groups removed.

3.4. Concluding Remarks and Future Work

Initial studies have begun using both TFA and NH_4F in solution at the same time. Prior results from our lab indicate that this will require careful tuning otherwise it is possible to transform the aryl –OH's to carbonyls forming the quinone instead of the catechol due to the basicity of the fluorine. This was previously controlled by finding the right conditions for the deprotection. Initial studies are aimed at observing the deprotection when both TFA and NH_4F are present in excess. Once these initial studies have been completed the structure will be confirmed and if needed the ratios adjusted.

Once the synthesis of the cationic terpolymer is complete adhesion studies will be done. These studies, which will be considering the same variables that were investigated poly[(3,4-dihydroxystyrene)-*co*-styrene], will be exploring how cationic charge effects adhesion. This will be analyzed for both dry and underwater environments. The data will be compared back to poly[(3,4-dihydroxystyrene)-*co*-styrene] and will help deepen our understanding of what role charges play in underwater adhesion.

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CHAPTER 4. ADHESIVE DELIVERY: A STICKY SOLUTION

4.1. Introduction

Having developed poly[(3,4-dihydroxystyrene)-*co*-styrene] into an underwater adhesive and achieving significant results a further avenue was explored primarily that of formulating our adhesive system for commercial delivery. The biggest challenge to commercializing poly[(3,4-dihydroxystyrene)-*co*-styrene] is that in its current incarnation all of the testing and development has been done under controlled laboratory conditions (*1-4*). While variables such as time, temperature, salinity, can all be varied in the lab the one that is harder to emulate is physical application. Currently, poly[(3,4-dihydroxystyrene)-*co*-styrene] is dispensed in solution from a glass syringe, on a horizontal surface, using a needle that allows for fine control. This will not be the case in the majority of commercial purposes. Commercial adhesives typically are either a two component system that is mixed just prior to bonding, typically by hand, or a single component system. These commercial products served as one part of the inspiration for the design of our own system. The other main source of inspiration follows the path of poly[(3,4-dihydroxystyrene)-*co*-styrene] itself and is inspired by nature.

Mussels apply there glue using their foot (*5-9*) Figure 4.1. This foot seeks out a stable surface that it can lay the plaque upon. Once the mussel has found a suitable surface it extends its foot. The mechanism by which it is believed that mussels deposit the plaque is shown in Figure 4.1. This mechanism is similar to that of a suction cup in that it allows for the removal of some debris and water from the site which it is depositing the plaque. Mussels are not the only animal that uses this suction cup mechanism underwater. The other animals well known for this are the squid and octopus.

Squids tentacles are covered in suckers, these suckers play a valuable role in the life of a squid (*10*). They can be used to move about or remain in place in addition to being part used to hunt and eat other animals. The suckers are controlled by muscles in the tentacles and when stripped down to its most basic form it is that of a suction cup (*10*). Many groups have mimicked squid suckers for variety of purpose whether it be underwater adhesion (*11*), robotic arm design (*12*), nano-patterned films (*13*), or dental resins (*11*). This mechanism that is found not only in squids but mussels as well served in part as inspiration for the design and testing of a delivery system for poly[(3,4-dihydroxystyrene)-*co*-styrene].

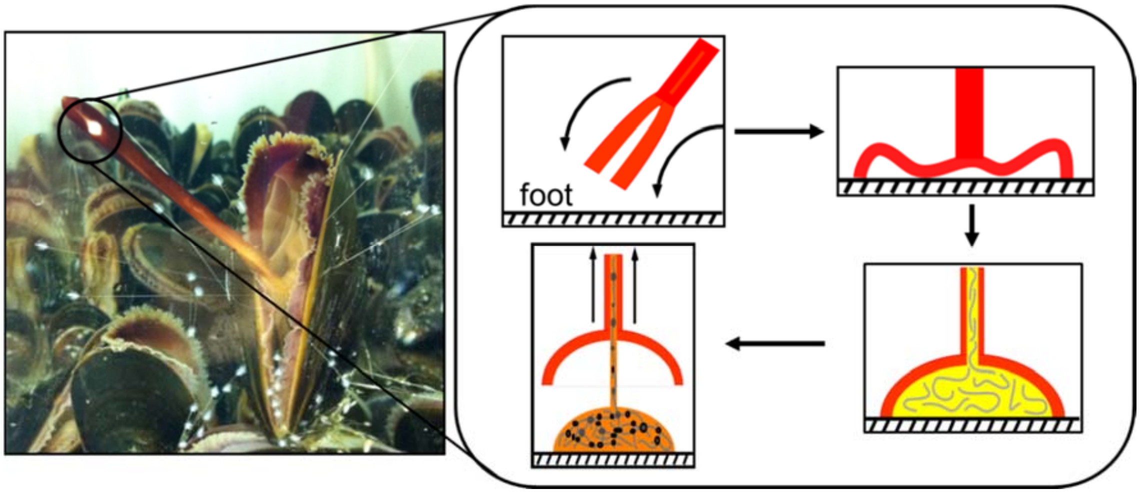

Figure 4.1. A mussel laying a plaque using its foot with a schematic of the delivery mechanism. Picture taken by Chelsey Del Grosso.

4.2. Material & Preparation

Dual body syringes were purchased from Pearson, a dental supply company. These came in different ratios of volumes either 1:1 or 1:4. A variety of mixing tips were also purchased and explored for use in this system. The squid suckers and the mussel foot mimic used were suction cups purchased from Vacmotion. A variety of sizes were purchased and tested as shown in Figure 4.2. Additionally, Teflon coated suction cups were also purchased from Vacmotion.

Poly[(3,4-dihydroxystyrene)-*co*-styrene] was made as previously described. The polymers once prepped were loaded into the dual body syringe by inverting the syringe removing the cap and loading the polymer through into one of the barrels of the syringe. The other barrel, depending on what was being tested, could be loaded with additional solvent, solvent plus cross-linker, or solvent, plus filler. This same loading strategy was applied when using commercial products. The commercial products already in dual barrel

Figure 4.2. Dual barrel syringe with a variety of suction cups and the complete assembly with a suction cup attached.

syringes where loaded into the dual barrel syringes that were being tested. In these cases the second body was the second component of the commercial adhesive system.

4.3. A Sticky Sucker

Initial testing was carried out with commercial systems in order to conserve polymer. The first commercial product tested was Mr. Sticky's underwater glue and Marine Loctite. Both of these glues are two component systems that had been tested previously as commercial comparisons to poly[(3,4-dihydroxystyrene)-co-styrene]. These glues were tested with the different suction cups in order to provide information on which suction cup was best suited to continue testing with.

The suction cups were tested by first placing them on the mixing tips as shown in Figure 4.2 and then trying to place them on a submerged adherend. This quick test automatically resulted in discarding three of the five missing tips for the size was either to large or too small and in the case of the red septum the design was not able to produce any void space. This left the clear and the black suctions cups shown in the left in Figure 4.2. Of these two the black suction cup was chosen to continue testing with as the pump-like design allowed for better control of the suction and creation of void space.

Having identified an appropriate system for testing, delivery of the two commercial products were compared to the results obtained previously during the poly[(3,4-dihydroxystyrene)-co-styrene] study in addition to using the assembly without the suction cup attached on the end as shown in Table 4.1. Similarly, poly[(3,4-dihydroxystyrene)$_{29\%}$-co-styrene$_{71\%}$] was also tested with the assembly with and without the suction cup present and compared back to previous results.

The data show that, unlike previously hypothesized, the suction cup does not under any circumstances provide a benefit to adhesion. In fact the opposite is observed that there is a sharp and noticeable decrease in adhesion while using the suction cup with both Marine Loctite and the biomimetic polymer having little to no measureable adhesion. This is largely due to material loss in the suction cup. The material is not deposited onto the substrate in a controlled manner. In an effort to minimize the material loss suction cups coated with Teflon were purchased and studied. These provided no noticeable benefit to the deposition of poly[(3,4-dihydroxystyrene)-*co*-styrene].

Table 4.1. Results of the dual barrel syringe assembly with and without the squid mimicking suction cup.

	Direct delivery	With suction cup	Without suction cup
Mr. Stickies	1.0 ± 0.3	0.07 ± 0.04	0.64 ± 0.30
Marine Loctite	0.6 ± 0.3	-	0.06 ± 5.5E^{-5}
Biomimetic Polymer	1.98 ± 0.33	-	0.33 ± 0.17

4.4. Ending Remarks and Future Directions

Additional issues arose when testing the delivery assembly. Loading the assembly proved challenging when using concentrated solutions. The poly[(3,4-dihydroxystyrene)-*co*-styrene] solutions also were observed to become more concentrated over time after being loaded into the assembly requiring mixing prior to use, Curing of the polymer was not observed as such. The main challenge for testing the delivery assembly is that of material loss. In each test of the assembly there is material lost mainly to the mixing tip and the suction cup. This material loss can be mitigated by truncating the tips shown in Figure 4.1, nearer to the syringe body. This results in a thicker stream of polymer being dispensed and only exacerbates the issue of control. It also provides less mixing of poly[(3,4-dihydroxystyrene)-*co*-styrene] with the contents of the second barrel of the syringe and yields lesser results.

Further testing of the syringe assembly is ongoing using a substitute for poly[(3,4-dihydroxystyrene)-*co*-styrene]. The substitute chosen for poly[(3,4-dihydroxystyrene)-*co*-styrene] is high molecular weight polystyrene due to its similar solubility's and properties, namely viscosity. Advances in the deposition and application technique are being made, as well insights into mixing with fillers, and cross-linkers. Once ideal conditions have been found transitioned back to the actual poly[(3,4-dihydroxystyrene)-*co*-styrene] system and adhesion test will be carried out and compared back to the previously benchmarked performance of this adhesive system.

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VITA

VITA

Michael Alexander North was born on February 13th of 1988 something which immediately caused regret as if he had waited a handful of hours he would have been a Valentine's Day baby and gotten free baby supplies. Raised in Merrill, Wisconsin Michael graduated from Merrill High School in 2006 a remarkable feat considering how many non-school related books he read. Somehow though Michael managed to worm his way into college and after two years at the University of Wisconsin Marathon County Michael achieved his Associates degree. During the course of these two years was when Michael decided to pursue a degree in chemistry. Michael left to finish his bachelors in chemistry at University of Wisconsin Eau Claire in the fall of 2008. In the following summer he began doing computational undergraduate chemistry research in the laboratory of Sudeep Bhattacharyya. Graduating with his bachelor's in 2011 Michael left the forested rolling hills of northern Wisconsin for the corn fields surrounding West Lafayette, Indiana and Purdue University. Upon his entry into Purdue University Michael quickly became stuck on the idea of joining Dr. Jonathan J. Wilker's laboratory. After joining, Michael's work focused on developing a biomimetic polymer system into one of the strongest underwater glues available. Having gained many wonderful experiences and valuable life lessons from Purdue University, Michael received his Doctor of Philosophy in Chemistry

PUBLICATION

Submitted for Publication

High Strength Underwater Bonding with Polymer Mimics of Mussel Adhesive Proteins

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Abstract

When it comes to underwater adhesion, shellfish are the true experts[1-3]. Mussels, barnacles, and oysters attach themselves to rocks with apparent ease. Yet our man-made glues often fail when trying to stick in wet environments. We are in need of biomimetic materials to both understand how bioadhesion works as well as gain access to properties not yet available[4]. In work describe here a copolymer system, poly(catechol-styrene)[5,6], was used to mimic mussel adhesive proteins. Parameters such as copolymer composition and molecular weight were examined to determine the key aspects of function. In doing so, findings that are both exciting and surprising came to light. Poly(catechol-styrene) may be the strongest underwater adhesive found to date, with performance exceeding that of established commercial products. Bonding even surpassed that of the reference biological system, live mussels. Adhesion was found to be stronger in salt water than deionized water. Such an unexpected result may contradict a proposal in which charged amino acids are thought to be key for the sticking of mussel proteins[7]. Taken together, these discoveries are providing insights on the function of biological systems in addition to creating new high performance materials.

Underwater adhesion presents several technical challenges[1]. When applied to submerged substrates, glues interact with water prior to forming adhesive bonds atop the surface or cohesive bonds within the bulk. Although man-made adhesives do not work well underwater, nature has been addressing such design constraints for eons. A trip to the beach will show rocks covered by organisms including oysters, barnacles, sea grasses, and tube worms, each sticking with adhesives[1-3]. The common blue mussel (*Mytilus edulis*) has gained a measure of fame by being the role model for our understanding of wet bonding (Fig. 1)[1-3]. This shellfish attaches upon depositing a mixture of proteins containing an atypical amino acid, 3,4-dihydroxyphenylalanine (DOPA) (Fig. 1)[1-3]. Cross-linking of these proteins generates cured glue. Despite such insights, we still do not understand how this system can work so well in an environment that tends to be particularly harsh toward adhesion. Furthermore, we have not yet been able to take what we have learned from biology and transition this knowledge into fully functioning biomimetic materials[4].

In recent years there has been a blossoming of material systems that mimic various aspects of mussel adhesive proteins[1-3,8-10]. Quite often synthetic polymers are used to substitute for the protein backbone and derivatives of catechol are appended to these chains for providing the cross-linking and adhesion chemistry of DOPA (Fig. 1)[5-7,9-18]. Notable findings have included hydrogels being developed with self-healing[19]. In terms of adhesion, dry bonding strengths of mussel mimicking polymers have been able to exceed that of long established commercial products including Super Glue[5]. Despite these advances, we are still lacking high strength bonding with synthetic materials when used underwater. We have also not developed much context for the performance of biomimetic materials in comparison to the biological counterparts. In work described here, a polymer system is shown to bond underwater with high strengths in bulk applications. Several aspects of polymer design were explored and, in the end, underwater bonding exceeded that achievable by the animals after which this material was modeled. In many regards the findings are surprising and serve to influence our understanding of how biology achieves underwater adhesion.

Prior studies have shown that the random copolymer poly[(3,4-dihydroxystyrene)-*co*-styrene] ("poly(catechol-styrene)") is a useful mimic of mussel adhesive proteins in terms of dry bonding performance (Fig. 1)[5,6]. Catechol groups pendant from a polystyrene host can represent, respectively, DOPA distributed throughout the polypeptide chains of mussel adhesive proteins[5,6]. The copolymers were synthesized on gram scales, thereby enabling bulk adhesion testing. Dry bonding of the polymer was appreciable, well into the Megapascal (MPa) range for lap shear joints between metal, plastic, and wood substrates[5,6]. How well might the dry bonding of this biomimetic system transfer to underwater applications? In terms of polymer composition and molecular weight, which derivatives should bring about the highest underwater bonding performance? Looking at the proteins found in a mussel's adhesive plaque does not provide too much help with regard to design. The DOPA content can range from 3% to 30% of all amino acids[20]. Molecular weights are as low as 6,000 and as high as 110,000 grams/mole[21].

In order to explore underwater adhesion, a logical starting point was a polymer of ~33% 3,4-dihydroxystyrene and ~67% styrene, given prior data on the poly[(3,4-dihydroxystyrene)$_{33\%}$-*co*-(styrene)$_{67\%}$] composition yielding maximum dry adhesion[6]. Molecular weight can have a major impact upon adhesion, with shorter chains providing surface wetting, yet longer molecules being best at bringing about polymer-polymer interactions for cohesion[16,22,23]. Poly(catechol-styrene) of several different molecular weights was made here by changing the ratio of *n*-butyl lithium polymerization initiator to monomers in the reaction feed (Extended Data Table 1). The catechol content of these polymers was held between 27% and 33% (Extended Data Table 1).

To test underwater bonding, polished aluminum substrates were submerged into a tank of artificial sea water (Fig. 1). Poly(catechol-styrene) was dissolved into chloroform and the solution deposited onto a substrate. The choice of chloroform was dictated by needing a solvent denser than water such that the adhesive formulation did not float up and off the substrate. A second piece of aluminum was then placed atop the first to create a lap shear joint. After addition of a weight to hold the substrates together during a cure period, the assembly was removed from the tank and pulled apart immediately by a materials testing system to quantify bonding. Maximum force at failure was divided by substrate overlap area to provide adhesion values (in MPa). Data in Fig. 2a show a strong dependence of adhesion upon polymer molecular weight. Performance peaked at ~85,000 grams/mole. This point is likely where surface adhesive and bulk cohesive forces achieve an optimal balance. Analogous data for dry bonding differ somewhat, with no obvious peak and increasing molecular weights correlating to higher adhesion even over 100,000 grams/mole [16].

The degree of cross-linking can also sway an adhesive-cohesive balance. Oxidized catechols (i.e., semiquinone or quinone) bring about cross-linking to generate cohesive bonds within the material[11,24]. However, it is the reduced form of the DOPA catechol ring that is responsible for surface adhesive contacts[3]. Consequently, the amount of catechol within the polymer will influence overall adhesion. Figure 2b presents a study in which several poly(catechol-styrene) derivatives were made, each with differing amounts of the catechol-containing 3,4-dihydroxystrene monomer (Extended Data Table 2). Polymer molecular weights were maintained at 75,000 - 101,000 grams/mole in keeping with the results from Fig. 2a for optimal bonding. Maximum underwater adhesion was found with a polymer of ~22% 3,4-dihydroxystyrene and ~78% styrene (Fig. 2b). Here, too, results for underwater bonding differed somewhat versus dry conditions, which maximized at ~33% 3,4-dihydroxystyrene and ~67% styrene[6].

Making comparisons between adhesion data is often difficult, given variations in several parameters including substrate choice, cure conditions, joint type, and testing methods. However, we can gain some context for how poly(catechol-styrene) compares to prior efforts. Bulk lap shear joints between aluminum substrates with charged catechol-containing polymers have been reported at 0.35 MPa for polyoxetanes in humid conditions with partial drying[15]. When in a coacervate phase of a polyanion condensed with Ca^{2+} cations, strengths up to 1.2 MPa were found[14]. Neutral catechol-containing polyvinylpyrrolidone applied to wetted glass and then cured underwater was at 1.3 MPa[17] and a polyacrylate between wet glass bonded at 1.6 MPa[25]. Beyond mussel mimicking systems is a metal complex guest and macrocycle host, each surface grafted onto silicon, yielding up to 1.1 MPa underwater[26]. A light cured bisphenol-acrylate adhered aluminum underwater at 1.2 MPa[27]. Improvements in bulk adhesive performance are typically gradual, with a factor of 2X enhancement being quite significant. With strengths up to ~3 MPa, bulk underwater adhesion with poly(catechol-styrene) was quite appreciable.

In order to provide benchmarks for direct comparisons, bonding was carried out with a range of commercial glues including common adhesives and specialty materials billed for wet applications. All glues were applied underwater with constant conditions including quantity of adhesive, cure time, cure temperature, and substrate type. Figure 3 provides data indicating that poly(catechol-styrene) outperformed every product tested, usually by quite large margins. Standard adhesives such as Elmer’s Glue-All (polyvinyl acetate) and Super Glue (ethyl cyanoacrylate) failed to bond at even modest levels, likely a result of not being able to cure underwater or the water inducing curing too rapidly to allow interaction with the substrates, respectively. We now have what appears to be the strongest underwater adhesive reported to date.

For providing a broader context of underwater bonding capability, poly(catechol-styrene) adhesion on a range of different substrates was compared to some of the strongest commercial glues from Fig. 3. Extended Data Table 3 shows that these selected commercial products performed best with polyvinyl chloride (PVC), etched aluminum, and sanded steel substrates. On polytetrafluoroethylene (Teflon), wood (red oak), and polished aluminum, poly(catechol-styrene) displayed the highest adhesion. With Teflon, only the biomimetic polymer and a single commercial product provided any bonding at all. For wood, poly(catechol-styrene) was the single system capable of creating a measurable bond underwater. Given that the commercial glues have been around for up to decades, we are excited to report superior performance for a relatively young biomimetic system.

A new material that can, at times, outperform established products is quite exciting[4]. Perhaps even more challenging is direct comparison of a biomimetic system against the true biological counterpart. Live mussels (Fig. 4a) were placed atop sheets of polished aluminum for deposition of their adhesive. Using an established method (Fig. 4b) and several animals, adhesive plaques were pulled up from the surfaces until failure[28]. Average mussel adhesion in this tensile mode was 0.13 ± 0.01 MPa. For a direct comparison, polished aluminum rods were held under salt water and then bonded together into tensile joints using poly(catechol-styrene) (Fig. 4c). The rods were pulled apart (Fig. 4d) to reveal bonding at 2.2 ± 0.9 MPa, a 17-fold increase over the animal's adhesion.

Synthetic systems can mimic nature, but seldom outperform biological materials. Despite a large degree of effort, biomimetic properties similar to, for example, sea shells, bone, or wood have not yet been achieved[4]. Our work described here has been aimed at creating the strongest underwater glue possible. A living mussel, by contrast, need only attach strongly enough to deter the forces exerted by waves and predators. Perhaps such differences in end goals can, at least partially, explain how poly(catechol-styrene) outperformed mussel adhesive.

Several recent reports have been contributing to our understanding of how mussels bond to rocks so well. Catechols may have a special ability to drill down through surface-bound waters for enabling wet attachment. Having two, adjacent alcohol groups might allow for cooperative binding, analogous to an entropic "chelate effect"[29]. Hydrogen bonding and metal chelation at the substrate appear likely to be contributing surface adhesion[29]. Oxidative cross-linking generates cohesive forces[2,24,30]. Cationic charges within mussel adhesive proteins have been proposed recently to aid this bonding in salt water[7]. Positively charged amino acids could help outcompete surface-bound cations such as sodium, thereby allowing proteins to gain access onto mildly anionic surfaces including rocks[7]. In order to address potential roles for charges[7,12] and salts[7,12,18,19,27], we examined the bonding of poly[(3,4-dihydroxystyrene)$_{28\%}$-*co*-(styrene)$_{72\%}$] (M_w = 95,000 grams/mole) in deionized water (pH = 7.9) and found a value of 0.4 ± 0.1 MPa. When the same experiment was carried out under artificial sea water (pH = 7.9), adhesion was at 1.8 ± 0.2 MPa. Note that poly(catechol-styrene) is a neutral polymer and we might expect improved adhesion under deionized versus salt water. This unexpected finding could be a function of the current study using a bulk, macroscopic adhesion method versus prior efforts examining interactions on the nanometer scale[7]. Nonetheless, such data indicate that disruption of charges atop submerged surfaces may not be of primary importance for adhesion in the seas.

Results presented here show that man-made materials can, indeed, bring about quite significant underwater adhesion. A biomimetic copolymer yielded the strongest underwater bonding found to date. Furthermore, in a rare instance of biomimetics, this synthetic system outperformed the reference biological counterpart. Such findings are helping to reveal how mussels manage attachment within their salty environment. Shellfish, the true masters of wet bonding, continue to teach us the secrets of adhesion.

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Author Contributions

M.A.N. was involved in experimental design and carried out the polymer synthesis and adhesion described herein. C.A.D.G. cared for the animals and measured their adhesion. J.J.W. oversaw the project. The manuscript was written by M.A.N., C.A.D.G., and J.J.W.

Author Information

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Online Content

Methods, along with additional Extended Data items, are available in the online version of the paper; references unique to these sections appear only in the online paper.

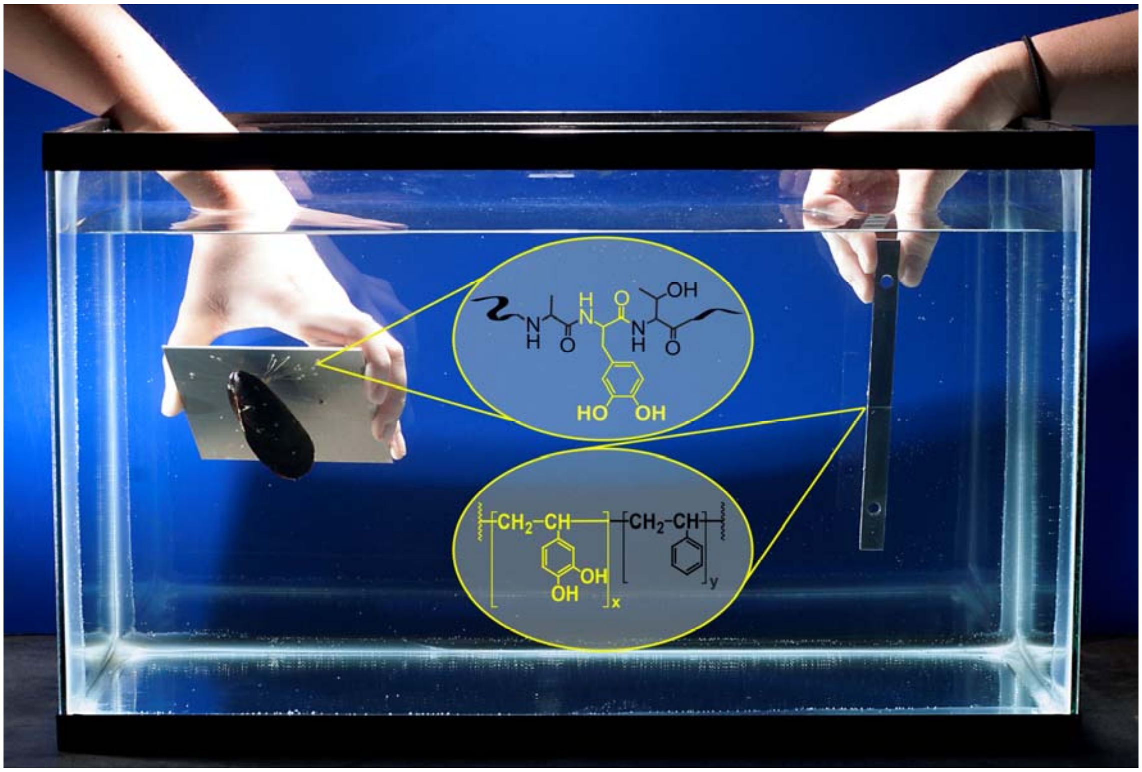

Figure 1. Underwater bonding of a marine mussel and poly(catechol-styrene). The structure of 3,4-dihydroxyphenylalanine (DOPA) in a protein and poly[(3,4-dimethoxystyrene)-*co*-(styrene)] are shown. The mussel is attached to a piece of aluminum. The polymer is forming a lap shear joint between two aluminum substrates. Catechol-containing components are highlighted in yellow.

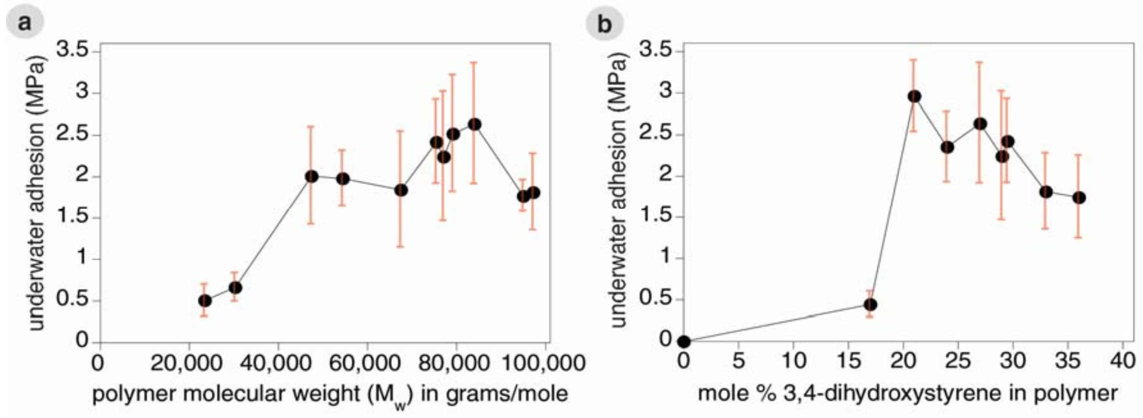

Figure 2. Polymer influences upon underwater bonding. a) Adhesion as a function of molecular weight for poly(catechol-styrene). **b)** Changes in adhesion with varying catechol content of the polymers.

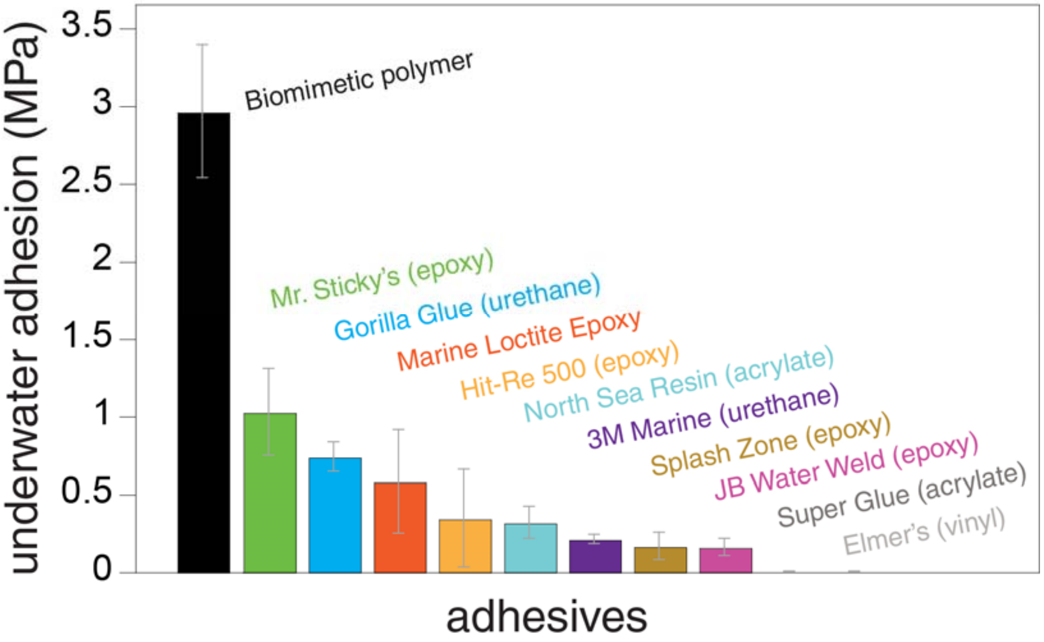

Figure 3. Underwater bonding of poly(catechol-styrene) compared to commercial products. Lap shear joints were made between polished aluminum substrates.

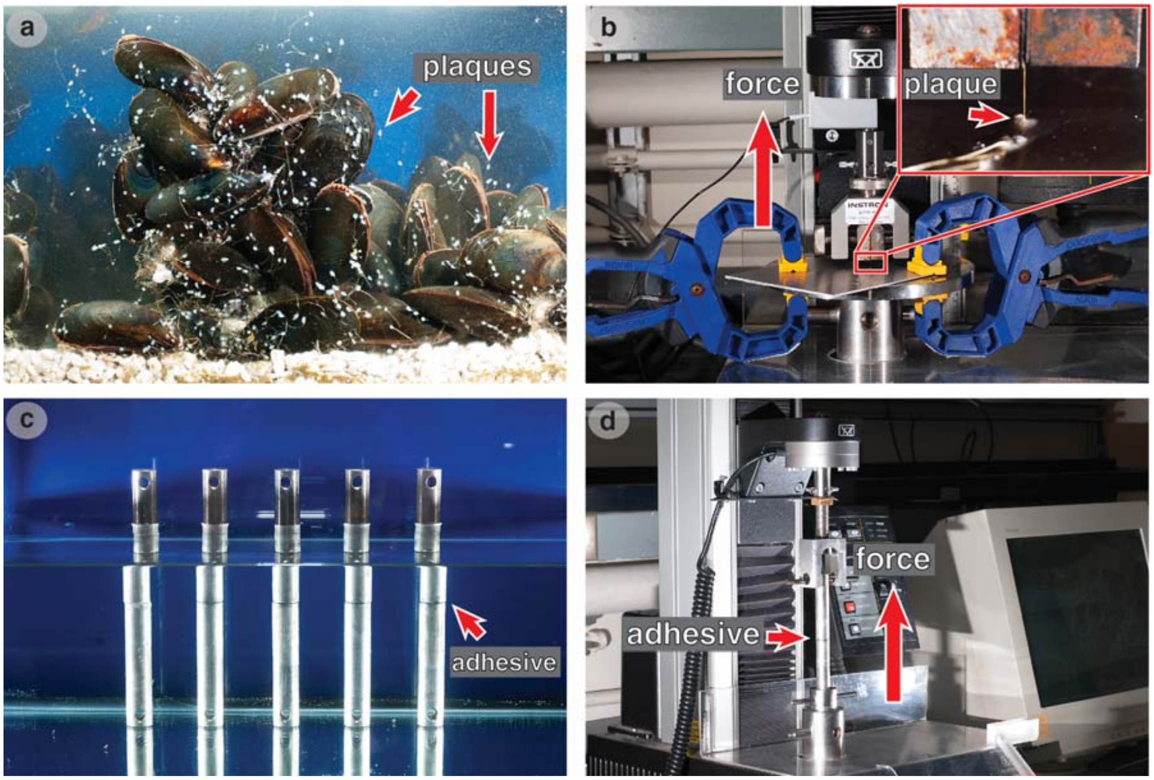

Figure 4. Comparing the adhesion of live mussels and a biomimetic system. **A)** Mussels seen depositing adhesive plaques onto the side of an aquarium tank as well as upon each other. **B)** Testing the adhesion strength of mussel adhesive. The inset shows how grips pull up on the thread while the adhesive plaque is bound to the substrate. **C)** Aluminum rods held underwater and bonded together in a tensile joint with poly(catechol-styrene). **D)** Measuring adhesion after an underwater cure. The substrates were first underwater, poly(catechol-styrene) was applied, both substrates were joined together, and the assembly cured for 72 hours. The joint was then removed from the water for immediate testing.

Methods

Polymer synthesis

The poly[(3,4-dimethoxystyrene)-*co*-(styrene)] precursor copolymers were synthesized using a prior method [6]. Briefly, styrene, 3,4-dimethoxystyrene and toluene were combined in a flame-dried Schlenk flask. The flask was then cooled in a dry ice/isopropanol bath. After 10 minutes, *n*-butyl lithium was added to initiate polymerization. The reaction mixture was kept on dry ice for 8 hours and then allowed to warm up to room temperature gradually. After 24 hours of total time, the reaction was quenched and the polymer precipitated by addition of methanol. The polymer was redissolved into chloroform and then reprecipitated with methanol. This purification procedure was repeated three times. The polymer was dried via rotary evaporator and placed under vacuum overnight.

Polymer deprotection

Poly[(3,4-dimethoxystyrene)-*co*-(styrene)] was converted to poly[(3,4-dihydroxystyrene)-*co*-(styrene)] by dissolving the former into dichloromethane in a flame-dried Schlenk flask [6]. The flask was placed into an ice bath for 10 minutes after which boron tribromide was added. This reaction proceeded overnight, was quenched with methanol, and allowed to stir for 15 minutes. The mixture was then poured into 1% hydrochloric acid and stirred for 15 minutes. After being allowed to settle, the excess hydrochloric acid was decanted. This procedure was repeated 3 times. The solid was dissolved into dichloromethane and acetone then dried via rotary evaporator. The final white solid was placed under vacuum overnight.

Polymer characterization

Polymers were characterized primarily with proton nuclear magnetic resonance (^{1}H NMR) spectroscopy and gel permeation chromatography (GPC). The ^{1}H NMR spectra were recorded on a Varian Inova-300 MHz spectrometer and provided compositions. Gel permeation chromatography was performed in THF mobile phase on a Polymer Laboratories PLC-GPC20 to yield molecular weights (M_n and M_w) and polydispersity indices (PDI's).

Water preparation

Artificial sea water was prepared using Marine Environment dual phase formula and reverse osmosis water to a final salinity of 35 grams/liter. Deionized water was prepared using a Barnstead Nanopure Infinity Ultrapure water system with a final resistivity of 18 MΩ-cm. All water was prepared immediately prior to use. Deionized water was at pH = 8.0 directly from the purifier and at pH = 7.9 after 24 hours. The salt water was pH = 7.9 at both the beginning and end of the 24 hour experimental periods.

Lap shear adhesion testing

Lap shear adhesive bonding was carried out with a modified version [6] of the ASTM D1002 standard method [31]. Derivatives of poly[(3,4-dihydroxystyrene)-*co*-(styrene)] were dissolved at 0.3 g/ml in chloroform with 45 µL dispensed onto each completely submerged substrate. An additional 15 µL of chloroform was then deposited. Another substrate was placed on top of the first to form a lap shear joint of 1.2 cm x 1.2 cm. The bonds were cured at room temperature for 24 hours, completely submerged.

Samples were then removed from the water and measured immediately on an Instron 5544 materials testing system. Measurements used a 2,000 N load cell and a crosshead speed of 2 mm/min. The maximum force at joint failure divided by the overlap area provided the adhesion strength. Each sample was tested a minimum of 5 times and averaged. The molecular weight and catechol percent graphs of Figure 2 show averages of 10 samples. Error bars indicate 90% confidence intervals.

Preparation of Substrates

Substrates were fabricated by methods described previously [5,6]. Briefly, aluminum, type 6061 T6, was purchased and prepared either by mirror polishing with Mibro no. 3 and Mibro no. 5. polish or an ASTM D2651-01 method for adherend cleaning [32]. Red oak was purchased locally and had a surface roughness equivalent to that of treatment with 220 grit sandpaper. Steel adherends were sanded with 50 grit sandpaper prior to testing and then washed with ethanol, acetone, and hexanes. Teflon (PTFE) and PVC were obtained from Rideout Plastics.

Testing of commercial adhesives

Eleven different commercial glues were tested underwater using similar conditions to poly[(3,4-dihydroxystyrene)-*co*-(styrene)]. Each product was measured 5 times using a mass of 13.5 mg to match the mass of poly[(3,4-dihydroxystyrene)-*co*-(styrene)] in each trial (0.3 g/ml and 45 μL). Drying experiments noted no significant loss of mass or solvent from any of the commercial glues. Samples were cured for 24 hours while underwater and then tested immediately.

Animal handling

Blue mussels (*Mytilus edulis*) were maintained in an aquarium system described previously [28], with growth conditions of 4 °C, 35 grams/liter salinity, and constant aeration. Each mussel was held in place with a rubber band on one 10 x 10 cm polished aluminum panel. The adhesive plaques of nine adult mussels were examined.

Live mussel adhesion data collection

Removal force was collected on an Instron 5544 materials testing system. Adhesion testing was carried out three days after placement of mussels and panels into the aquarium. Three separate trials were conducted whereby a total of 9 animals were examined to yield 48 plaques. Adhesion measurements were all averaged per animal. These average values per animal were then averaged to get an overall mean adhesion measurement. This method minimizes the effect of one shellfish biasing data too much by providing a different number of plaques as well as particularly weak or strong bonding.

Tensile adhesion testing of polymers

Polished aluminum rods of 1.5 cm diameter were completely submerged underwater. Poly[(3,4-dihydroxystyrene)-*co*-(styrene)], 45 µL at 0.3 g/mL in chloroform, was applied to one adherend and overlapped with the second rod. These joint assemblies cured for 3 days underwater in order to mimic conditions of the live mussel testing. Ten samples were measured and averaged. The error provided is a 90% confidence interval.

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Extended Data for:

High Strength Underwater Bonding with Polymer Mimics of Mussel Adhesive Proteins

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Extended Data Table 1.	Polymer synthesis and characterization data for the molecular weight study
Extended Data Table 2.	Polymer synthesis and characterization data for the polymer composition study
Extended Data Table 3.	Underwater adhesion of poly(catechol-styrene) compared to commercial glues on several substrates

Extended Data Table 1. Polymer synthesis and characterization data for the molecular weight study. These polymers were used for the experiments in Figure 2a.

feed		final polymer				
3,4-dimethoxystyrene feed (mole %)	styrene feed (mole %)	final 3,4-dimethoxystyrene content (mole %)	final styrene content (mole %)	M_n (g/mole)	M_w (g/mole)	PDI
41	59	31	69	16,000	23,000	1.4
41	59	31	69	19,000	30,000	1.6
41	59	33	67	29,000	47,000	1.6
40	60	27	73	37,000	54,000	1.5
40	60	28	72	50,000	67,000	1.3
42	58	29	71	49,000	75,000	1.5
40	60	29	71	52,000	77,000	1.5
45	55	27	73	56,000	79,000	1.4
40	60	27	73	59,000	84,000	1.4
40	60	28	72	63,000	95,000	1.5
30	70	33	67	60,000	97,000	1.6

Extended Data Table 2. Polymer synthesis and characterization data for the polymer composition study. These polymers were used for the composition data in Figure 2b.

feed		final polymer				
3,4-dimethoxystyrene feed (mole %)	styrene feed (mole %)	final 3,4-dimethoxystyrene content (mole %)	final styrene content (mole %)	M_n (g/mole)	M_w (g/mole)	PDI
n/a(1)	n/a(1)	0	100	97,000	101,000	1.0
16	84	17	83	67,000	96,000	1.4
30	70	21	79	63,000	84,000	1.3
41	59	24	76	67,000	97,000	1.4
40	60	27	73	59,000	84,000	1.4
40	60	29	71	52,000	77,000	1.5
42	58	30	70	49,000	75,000	1.5
30	70	33	67	60,000	97,000	1.6
40	60	36	64	53,000	76,000	1.4

1) This polymer was purchased from Sigma-Aldrich.

Extended Data Table 3. Underwater adhesion of poly(catechol-styrene) benchmarked against commercial glues. Lap shear joints between several substrate types were examined.

adhesive	substrate					
	polished aluminum	etched aluminum	sanded steel	Wood	PVC	Teflon
Mr. Sticky's (epoxy)	1.0 ± 0.3	0.2 ± 0.1	0.4 ± 0.1	0	3.0 ± 0.6	0.1 ± 0.1
Marine Loctite Epoxy	0.6 ± 0.3	0	0.2 ± 0.1	0	2.0 ± 0.5	0
3M Marine Sealant (urethane)	0.20 ± 0.03	0.10 ± 0.02	0.2 ± 0.1	0	1.0 ± 0.3	0
North Sea Resin (acrylate)	0.3 ± 0.1	0	0.2 ± 0.1	0	0	0
Gorilla Glue (urethane)	0.7 ± 0.1	0.4 ± 0.2	0.5 ± 0.1	0	3.0 ± 0.6	0
Biomimetic polymer	**3.0 ± 0.4**	**0.2 ± 0.1**	**0.10 ± 0.02**	**0.20 ± 0.02**	**0.4 ± 0.1**	**0.3 ± 0.1**