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Effect of Nitro-Functionalization on the Cross-Linking and Bioadhesion of Biomimetic Adhesive Moiety

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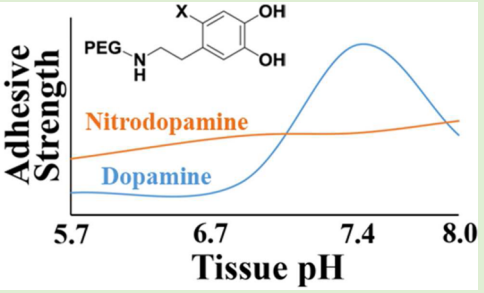
Supporting Information

ABSTRACT: Dopamine mimics the exceptional moisture-resistant adhesive properties of the amino acid, DOPA, found in adhesive proteins secreted by marine mussels. The catechol side chain of dopamine was functionalized with a nitro-group, and the effect of the electron withdrawing group modification on the cross-linking chemistry and bioadhesive properties of the adhesive moiety was evaluated. Both nitrodopamine and dopamine were covalently attached as a terminal group onto an inert, 4-armed poly(ethylene glygol) (PEG-ND and PEG-D, respectively). PEG-ND and PEG-D exhibited different dependence on the concentration of $NaIO_4$ and pH, which affected the curing rate, mechanical properties, and adhesive performance of these biomimetic adhesives differently. PEG-ND cured instantly and its bioadhesive properties were minimally affected by the change in pH (5.7−8) within the physiological range. Under mildly acidic conditions (pH 5.7 and 6.7), PEG-ND outperformed PEG-D in lap shear adhesion testing using wetted pericardium tissues. However, nitrodopamine only formed dimers, which resulted in the formation of loosely cross-linked network and adhesive with reduced cohesive properties. UV−vis spectroscopy further confirmed nitrodopamine's ability for rapid dimer formation. The ability for nitrodopamine to rapidly cure and adhere to biological substrates in an acidic pH make it suitable for designing adhesive biomaterials targeted at tissues that are more acidic (i.e., subcutaneous, dysoxic, or tumor tissues).



INTRODUCTION

The performance of most man-made adhesives and coatings is significantly compromised in the presence of moisture, which has traditionally been treated as a surface contaminant that needs to be removed to prevent the formation of a weak boundary layer.[1] In many applications, and particularly those in the medical field, there is a high demand for the development of moisture-resistant adhesives. Marine animals, such as common blue mussels, secrete remarkable underwater adhesives that allow these organisms to anchor to surfaces in turbulent, intertidal zones.[2,3] 3,4-Dihydroxyphenylalanine (DOPA) is found in large abundance in these adhesive proteins and DOPA is responsible for both the rapid curing of the adhesive and for interfacial binding.[4,5] Chemical modification of inert, synthetic polymers with DOPA and its derivatives (e.g., dopamine) has led to the development of various adhesive biomaterials for soft tissue repair,[6,7] drug delivery,[8] and antifouling coatings.[9−11]

The catechol side chain of DOPA is a unique and versatile adhesive molecule capable of binding to both organic and inorganic surfaces through either covalent attachment or strong reversible bonds. When catechol is oxidized to form highly reactive quinone, it participates in intermolecular covalent cross-linking leading to the rapid hardening of catechol-containing adhesives[12] and reacts with functional groups (i.e., $-NH_2$, $-SH$) found on biological substrates resulting in strong interfacial binding.[13,14] Additionally, catechols form strong reversible bonds with metal oxides[13,15] and ions,[16,17] with binding strengths that are strongly dependent on pH.[18−20] However, our lab recently reported that the oxidative cross-linking of dopamine was strongly affected by pH and its adhesive performance was severely compromised under mildly acidic physiological pH (i.e., 5.7−6.7).[21]

Similar to marine mussels, sandcastle worms secrete DOPA-containing adhesive proteins that these organisms utilize to cement sand fragments into a tubed-shaped dwelling.[22] The catechol side chain in the sandcastle worms' adhesive is further modified with an electron withdrawing chloro-functional group (2-chloro-4,5-dihydroxyphenylalanine), which was proposed to be a natural adaptation to increase interfacial binding strength.[23] Similarly, nitro-substituted catechol has also been found to form complexes with metal oxides that are more stable than unsubstituted catechols.[24−26] Functionalizing the catechol side chain with an electron withdrawing group (EWG) lowers the dissociation constants of the catechol hydroxyl groups (pK_a = 6.6 and 11 for 4-nitrocatechol compared to pK_a = 9.2 and 14 for catechol),[27] which may have resulted in enhancing the interfacial binding strength.[28] Although the effect of nitro-group modification on the adsorption of catechol to inorganic surfaces has been the subject of numerous investigations, its effect on

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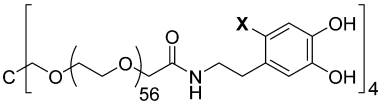
intermolecular cross-linking and adhesion to biological tissues has yet to be determined. Recently, Shafiq et al.[29] demonstrated that polyethylene glycol (PEG) end-capped with nitrodopamine undergoes oxidation-mediated curing. However, the mechanism and rate of oxidation-mediated cross-linking and bioadhesion were not a part of this study.

Here, we prepared branched, 4-armed PEG end-capped with either nitrodopamine or dopamine (PEG-ND and PEG-D, respectively; Scheme 1). Due to the inert and highly predictable nature of PEG, the observed changes in physical, mechanical, and adhesive properties can be fully attributed to the effect of nitro-group functionalization on the cross-linking chemistry and bioadhesion of the biomimetic dopamine adhesive. Specifically, we focused on the effect of changing pH and oxidant concentration on the curing rate, mechanical, and bioadhesive properties of PEG-ND adhesive and correlated these results with spectroscopy data to gain insight into the cross-linking mechanism of nitrodopamine.

Scheme 1. Chemical Structure of PEG-ND (X = NO_2) and PEG-D (X = H)



EXPERIMENTAL SECTION

Materials. Sodium phosphate monobasic monohydrate, sodium phosphate dibasic anhydrous, and sodium periodate ($NaIO_4$) were purchased from Acros Organics (Fair Lawn, New Jersey). Phosphate buffer saline (PBS, 1×) was from Fisher Scientific Co. (Pittsburgh, Pennsylvania). Fresh bovine pericardium was purchased from Sierra for Medical Science (Whittier, California). Pericardium tissues were cut into the desired dimension (2.5 cm × 2.5 cm), incubated in buffer solution of a desired pH (10 mM sodium phosphate buffer adjusted to a pH of 5.7, 6.7, 7.4, or 8.0) for 2 days and kept frozen until testing.[21] pH treatment had no impact on the tensile modulus of the tissue substrate (Table S1). PEG-ND[29] and PEG-D[21] were prepared using a previously published method using 4-armed 10k Da *N*-hydroxysuccinimide ester activated poly(ethylene glycol) purchased from JenKem USA, Inc. (Allen, Texas).

Formation of Hydrogel. The hydrogels were formed by mixing separately dissolved solutions of the polymer and $NaIO_4$ (both at double the final concentration) in 10 mM sodium phosphate buffer adjusted to a pH of 5.7, 6.7, 7.4, or 8.0. The final concentration of the polymer was kept at 75 mg/mL, while the $NaIO_4$ concentration was kept at a molar ratio of 0.25−1.5 relative to dopamine or nitrodopamine ($NaIO_4$ concentration of 14.5−87.0 mM). The cure time was determined when the polymer mixture ceased flowing in an inverted vial containing the fluid.[12,21]

Determination of Molecular Weight between Cross-Links ($\bar{M}_c$). PEG-ND hydrogels were characterized by the determination of the average molecular weight between cross-links ($\bar{M}_c$), as determined from equilibrium swelling data and application of the modified Flory−Rehner equation.[30] Hydrogels were allowed to cure for 24 h and cut into 1 cm diameter discs using a biopsy punch. Each hydrogel disc was weighed to determine its relaxed mass (M_r) before it was submerged in sodium phosphate buffer (pH = 7.4) for 24 h to find its swollen mass (M_s). The hydrogel swelled by a factor of 2 to 5 (Table S2). The dried mass (M_d) was found after the sample was dried under vacuum for at least 2 days. From the measured weight of the hydrogels, the densities of PEG (1.123 g/cm^3)[31] and water (1 g/cm^3) were used to find the volume of the gels in each state (V_r, V_s, and V_d in the relaxed, swollen, and dried state, respectively). The polymer volume fractions in the relaxed (v_r) and swollen (v_s) states were calculated using the following two equations:[32]

$$v_r = \frac{V_p}{V_r} \tag{1}$$

$$v_s = \frac{V_p}{V_s} \tag{2}$$

where V_p is the volume of PEG in the bulk hydrogel and is equal to V_d. $\bar{M}_c$ was calculated by the following equation:[30]

$$\frac{1}{\bar{M}_c} = \frac{2}{M_n} - \frac{\ln(1 - v_s) + v_s + \chi v_s^2}{\rho_p V_{H_2O} v_r \left[\left(\frac{v_s}{v_r}\right)^{1/3} - \frac{v_s}{2v_r}\right]} \tag{3}$$

where M_n is the starting molecular weight (MW) of PEG-ND, χ is the Flory−Huggins parameter for PEG and water (0.462),[33] ρ_p is the density of PEG, and V_{H_2O} is the molar volume of water (18.1 mol/cm^3).

Oscillatory Rheometry. The viscoelastic properties of the adhesive hydrogels were characterized with an oscillatory rheometer (HR-2, TA Instruments, New Castle, DE). Frequency sweep (0.01−100 Hz at 10% strain) experiment was performed to determine both the storage (G') and loss (G'') moduli of the samples after they were allowed to cure overnight. Hydrogel discs (diameter = 20 mm, n = 3) were tested using parallel plates at a gap distance that is set at 85% that of the individual hydrogel thickness, as measured by a digital vernier caliper.

Lap Shear Adhesion Testing. A total of 5 μL each of 300 mg/mL PEG-ND and 56 mM $NaIO_4$ solutions in 10 mM sodium phosphate buffer adjusted to the desired pH were added to one end of a piece of bovine pericardium (2.5 cm by 2.5 cm). The final concentration of the PEG-ND and $NaIO_4$ were 150 mg/mL and 27.8 mM, respectively ($NaIO_4$/nitrodopamine molar ratio = 0.5). These solutions were mixed using the tip of a pipet and the adhesive joint was formed by placing the second piece of bovine pericardium over the first with 1 cm overlap. The adhesive joint was compressed with a 100 g weight for 10 min and further incubated in PBS (pH 7.4) at 37 °C for overnight. The samples were pulled to failure using a servohydraulic materials testing system (8872 Instron, Norwood, MA) at a rate of 5 mm/min, and the maximum load and displacement were recorded.[34] At least seven samples were tested per formulation.

Spectroscopic Evaluation of Dopamine and Nitrodopamine Oxidation. A total of 50 μM PEG-ND (200 μM nitrodopamine) was dissolved in 10 mM sodium phosphate (pH 5.7, 6.7, 7.4, and 8.0) and evaluated with or without the addition of 100 μM $NaIO_4$ (0.5 molar ratio relative to nitrodopamine). At a series of predetermined times, UV−vis spectra (200 to 700 nm; PerkinElmer Lambda35) of the solution were recorded at a scan rate of 960 nm/min using sodium phosphate buffer as the reference.

Statistical Analysis. Statistical analysis was performed using JPM Pro 9 software (SAS, Cary, NC). One-way analysis of variance (ANOVA) with Tukey-Kramer HSD analysis was performed for comparing means of multiple groups, while the student t test was used for comparing means of two groups. A p-value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

When the PEG-ND precursor solution was mixed with $NaIO_4$, the mixture immediately solidified to form a hydrogel network (Figure 1). The cure time drastically decreased with increasing $NaIO_4$ concentration. For pH 5.7, cure time reduced from 281 ± 25.8 s to around 3 s when $NaIO_4$/nitrodopamine molar ratio was increased from 0.1 to more than 0.5. Cure time also drastically decreased with increasing pH. Formulations buffered at pH 7.4 and higher cured instantly when activated with a $NaIO_4$/nitrodopamine molar ratio 0.25 or higher. The cure

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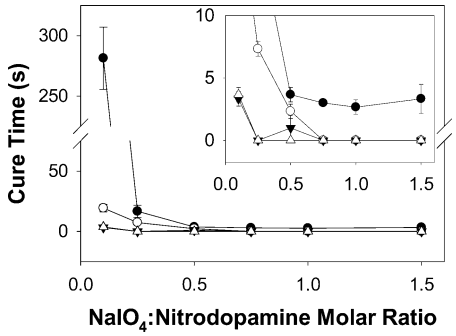

Figure 1. Cure time of PEG-ND as a function of $NaIO_4$/nitrodopamine molar ratio for hydrogels formulated with precursor solutions adjusted to a pH of 5.7 (●), 6.7 (○), 7.4 (▼), and 8.0 (△). The inset enlarges the cure time results under 10 s.

time of PEG-D also demonstrated a similar pH dependence.[21] However, PEG-ND demonstrated a significantly shorter cure time when compared to PEG-D, which cured within 30 s to 50 min at pH 7.4 (Figure S1). Under acidic conditions, the cure time for PEG-D is further retarded (6−70 min at pH 5.7).[21] PEG-ND also demonstrated a different $NaIO_4$ concentration dependence as compared to either dopamine or DOPA, where the shortest cure time occurred at a $NaIO_4$:catechol molar ratio between 0.5 to 1.[12,21] This suggests that nitrodopamine cross-linked via a different cross-linking mechanism than that of the unmodified catechols. Curiously, PEG-ND demonstrated an exponential decrease in cure time with increasing oxidant concentration similar to the response of PEG-DOPA cure rate under enzyme-mediate cross-linking.[12]

The equilibrium swelling behavior of PEG-ND hydrogel (Table S2) was used to determine the average molecular weight between cross-links ($\bar{M}_c$) of the polymer networks (Figure 2).

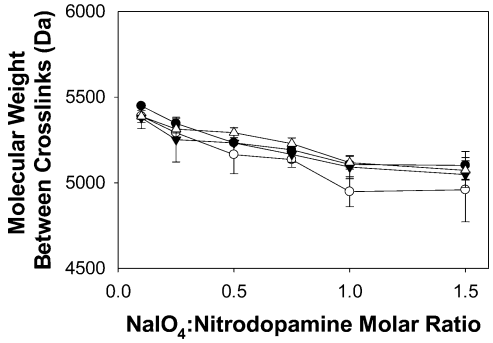

Figure 2. $\bar{M}_c$ as a function of $NaIO_4$/nitrodopamine molar ratio for hydrogels formulated with precursor solutions adjusted to a pH of 5.7 (●), 6.7 (○), 7.4 (▼), and 8.0 (△).

$\bar{M}_c$ is defined as the average molecular weight of the polymer chain between two consecutive junctions in a network and is inversely proportional to the cross-linking density and stiffness of the material.[35,36] Regardless of formulation pH, $\bar{M}_c$ decreased with increasing $NaIO_4$ concentration, indicating the formation of a more densely cross-linked network. Additionally, pH had no effect on the calculated $\bar{M}_c$ values as the calculated values were not statistically different. When compared to previously calculated $\bar{M}_c$ for PEG-D (2000−3000 Da),[21] values for PEG-ND (5000−5400 Da) were significantly higher (Figure S2). Both PEG-ND and PEG-D are prepared from a 10 kDa MW 4-armed PEG with four inert PEG chains of equal length (e.g., 2500 Da each) terminated either with a reactive nitrodopamine or dopamine moiety. The branched architecture of PEG forms a junction point when the terminal catechol groups dimerize (Scheme S1). In which case, the expected $\bar{M}_c$ value would be around 5000 Da, double that of the MW of each PEG arm. However, when the catechol forms oligomers with 3 or more catechol moieties, a new network junction is formed and the $\bar{M}_c$ value would average around 2500 Da (i.e., the MW of each PEG arm). The calculated $\bar{M}_c$ values suggest that the cross-linking of nitrodopamine predominately formed dimers. On the other hand, dopamine formed oligomers with higher numbers of repeats, similar to the behavior of DOPA, which was previously determined to form oligomers with the number of repeats as high as six.[12] The use of modified Flory−Rehner equation assumes the formation of an ideal network, where all the polymer chains in the network are elastically effective. Further studies will be required to verify unambiguously that nitrodopamine participates exclusively in dimerization.

Oscillatory rheometry was used to verify the formation of a hydrogel network and determine the viscoelastic properties of PEG-ND and PEG-D hydrogels (Figure 3). For both adhesives,

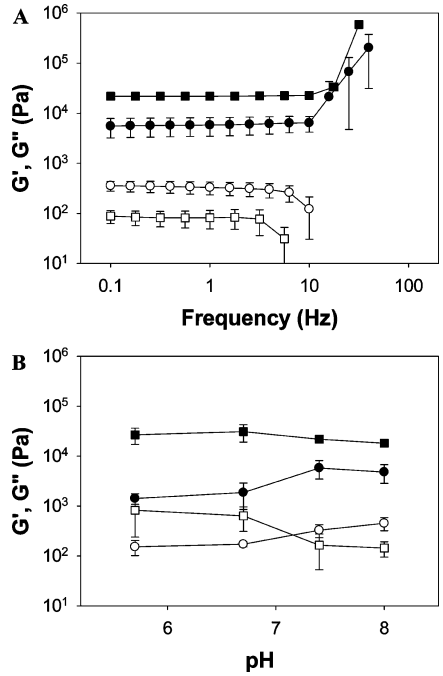

Figure 3. Rheological measurement of hydrogels (formulated at pH 7.4) during oscillatory frequency sweep (0.1−100 Hz, strain =0.1) (A) and values taken at a frequency of 1 Hz and a strain of 0.1 for hydrogels formulated at different pH levels (B). The symbols are G' (●) and G'' (○) for PEG-ND and G' (■) and G'' (□) for PEG-D; n = 3 for each measurement.

the measured storage modulus (G') values were independent of frequency (up until around 10 Hz) and the measured G' values were also significantly higher than those of the loss modulus (G'') values. These observations indicated the formation of covalently cross-linked networks.[15] G' values rose sharply for both PEG-ND and PEG-D gels at elevated frequencies. Long polymer chains between cross-links likely were not given the opportunity to rearrange themselves within the short time scale of the imposed deformation and these networks stiffened and exhibited elevated G' values.[37,38] The onset frequency for PEG-ND (10 Hz) occurred earlier than that of PEG-D (17 Hz),

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suggesting that PEG-ND hydrogel is more loosely cross-linked than its counterpart.[15] Similarly, G' value for PEG-D were also significantly higher (4−19 times higher) than those of PEG-ND for all the pH tested (Figure 3B), further confirming that PEG-D hydrogels were more densely cross-linked than PEG-ND. Even though both polymers were prepared with PEG macromolecules with the same architecture and MW, a drastically different cross-linking density between PEG-ND and PEG-D can be fully attributed to the difference in the degree of polymerization between the two catechol species.

Two lap shear adhesion experiments were performed to determine the effect of pH on the adhesive properties of PEG-ND and compare its performance with that of PEG-D adhesives. In the first experiment, hydrogel precursor solutions were buffered to a desired pH, while the tissue substrate was maintained at a pH of 7.4 (Figure 4A). Synthesis of catechol

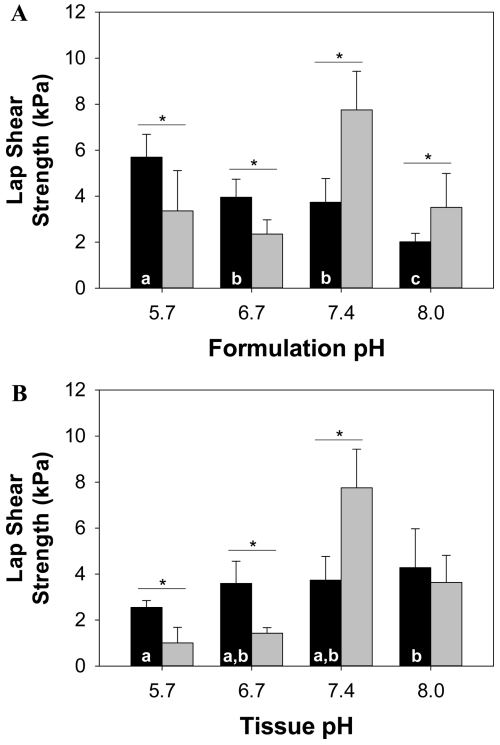

Figure 4. Lap shear adhesion test results performed with the precursor solutions adjusted to various pH levels using pericardium equilibrated at pH 7.4 (A) and the precursor solution buffered at pH 7.4 while using pericardium equilibrated at various pH levels (B). PEG-ND (black) and PEG-D (gray) were cured with a $NaIO_4$/catechol molar ratio of 0.5. PEG-ND formulations not linked by the same lower case letters (a−c) indicate statistical difference based on one-way ANOVA analysis ($p < 0.05$). The symbol * denotes statistical difference between PEG-ND and PEG-D results tested at the same pH as determined using student t test ($p < 0.05$). For (B), 2 out of 9 of PEG-D samples failed prior to testing for tissue buffered at pH 5.7. The adhesion data for PEG-D was obtained from published report.[21]

containing adhesive requires acid and base treatments[21,29,39,40] and this experiment simulates the effect of residual impurities from the synthesis of the adhesive polymer that could alter the pH of the adhesive formulation. Measured adhesive strength was the highest when PEG-ND was formulated at a pH of 5.7 and the adhesive strength of PEG-ND decreased with increasing pH. At an elevated pH, PEG-ND cured instantly (Figure 1). Under basic conditions, the cohesive cross-linking between nitrodopamine moieties were favored over interfacial cross-linking with the tissue substrate, resulting in reduced adhesive strength. On the other hand, a slower curing PEG-ND formulated at pH 5.7 likely yielded a balance of cohesive and interfacial cross-linking and a higher measured adhesive strength. When compared to PEG-D, PEG-ND formulated under mildly acidic conditions out performed PEG-D. Cross-linking of PEG-D was previously determined to be severely compromised under mildly acidic conditions.[21] At an elevated pH, the ability for PEG-D to form a densely cross-linked network with elevated cohesive properties likely contributed to enhanced lap shear adhesive strength. Additionally, PEG-D cured dramatically slower than that of PEG-ND, giving dopamine a chance to cross-link with tissue substrates leading to the higher adhesion strength at a pH of 7.4.

In the second adhesion experiment, pericardium substrates were equilibrated at various pH levels prior to testing while keeping the adhesive precursor solution buffered at pH 7.4 (Figure 4B). This experiment simulates the effect of applying adhesives to tissue with a pH that deviates from 7.4 (e.g., adhesion to subcutaneous tissue, dysoxic tissue during surgery, and tumor tissues with pH around 7 or less).[41−43] Measured adhesive strength for PEG-ND increased with increasing tissue pH. Nitrodopamine presumably reacted with nucleophiles found on tissue substrates (e.g., $-NH_2$ from lysine, −SH from cysteine, etc.), similar to the oxidation chemistry of DOPA.[18,19] Under acidic conditions, these nucleophilic groups are protonated (e.g., pK_a of ε-lysine ∼10), which reduces their ability to form interfacial bonds. Although cross-linking between nitrodopamine and these nucleophilic groups has not been previously reported, nitrodopamine with a free amine group undergoes intramolecular cyclization (Scheme S2),[44,45] indicating that adduct of a primary amine to the nitro-functionalized catechol is feasible. When compared to PEG-D, PEG-ND outperformed PEG-D under acidic conditions, while PEG-D adhered equally as well or better to tissue buffered at neutral to basic pH. Although there was a statistical difference within the PEG-ND adhesive strength, the variation in the measured values for different pH levels was much lower when compared to the variation of PEG-D, indicating that PEG-ND is less sensitive to changes in pH than PEG-D. Both PEG-D and PEG-ND outperformed a commercially available PEG-based sealant, CoSeal (Baxter, Inc., 0.63 ± 0.19 kPa, Table S3), tested at pH 7.4.

These two adhesive experiments clearly illustrate the importance of both the bulk cohesive and interfacial properties in the performance of an in situ curable adhesive. In our system, the catechol group is responsible for both cohesive and adhesive cross-linking. When PEG-ND was formulated at an increasing higher pH, the faster rate of nitrodopamine−nitrodopamine cross-linking limited the opportunity for nitrodopamine to form interfacial bonds (i.e., with tissue surface). This resulted in reduced lap shear strength when the adhesive was formulated at a basic pH. This pH-dependent trend differed from changes in the pH on the tissue surface. Decreasing pH on the tissue surface reduced the availability of the nucleophilic group for forming interfacial bonds with the adhesive, even though the bulk cohesive properties of the adhesive was unchanged. A balance in the bulk cohesive and interfacial properties is necessary for developing strong adhesives.

At pH 5.7, PEG-ND revealed two UV−vis absorbance peaks (310 and 355 nm) corresponding to the reduced form of

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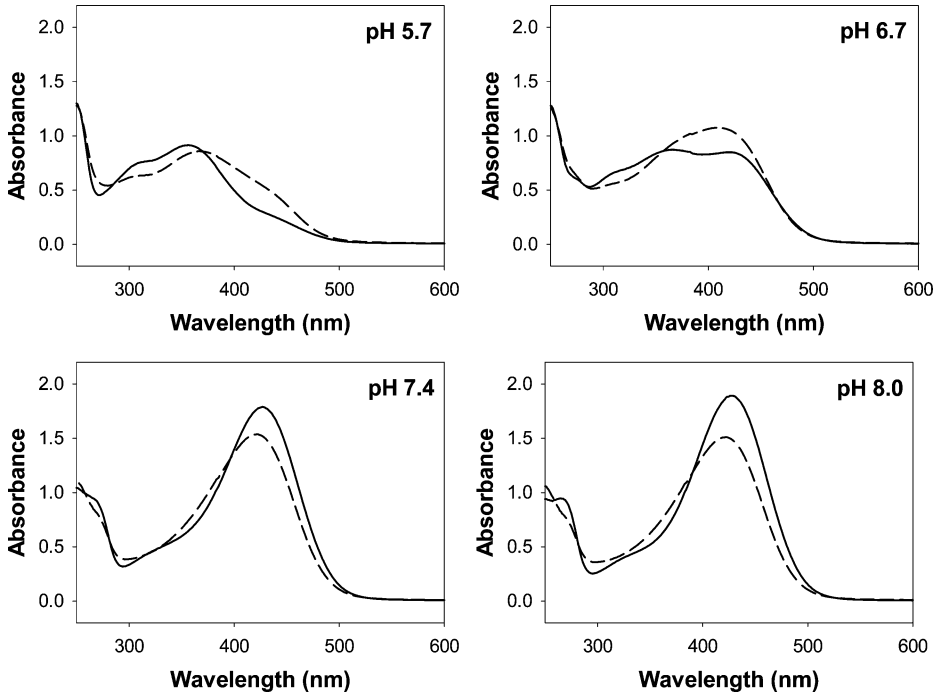
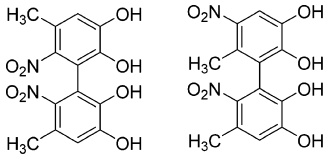


Figure 5. UV−vis spectrum of 50 μM of PEG-ND (200 μM nitrodopamine) without (solid line) and with (dashed line) 100 μM of $NaIO_4$. Solutions containing $NaIO_4$ were scanned immediately after the addition of the oxidant and no appreciable changes were observed for nearly 60 min.

nitrodopamine (Figure S3).[28] With increasing pH, the absorbance of these two peaks decreased, while two new peaks emerged (265 and 427 nm) corresponding to the deprotonation of one of the catechol −OH groups (or the formation of a semiquinone).[27] The dissociation constant of the first −OH group (pK_{a1}) of nitrodopamine has been found to be 6.5,[28] and PEG-ND showed features of all four peaks at pH 6.7, indicating the presence, and potentially a near stoichiometric ratio, of the nitrodopamine and its semiquinone. When $NaIO_4$ was added to PEG-ND, a new peak at 422 nm emerged, regardless of the pH tested (Figure 5). This peak corresponded favorably with dimers formed from oxidative cross-linking of 4-methyl-5-nitrocatechol (Scheme 2).[45] There

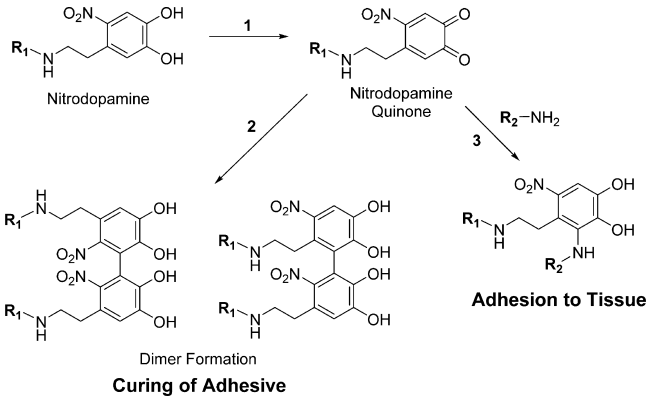
Scheme 2. Chemical Structures and Absorbance Maxima of Dimers Formed through Oxidative Cross-Linking of 4-Methyl-5-nitrocatechol[45]



was an increase in the intensity at 422 nm with increasing pH, indicating that dimerization is favored under basic conditions. Increased formation of nitrodopamine dimer corresponded to the observed increase in the measured G' values (Figure 3B) and reduction in $\bar{M}_c$values (Figure 2) as a result in an increased cross-linking density at an elevated pH. UV−vis spectra for the oxidized PEG-ND did not change when they were monitored for 60 min after the addition of $NaIO_4$, suggesting that the cross-linking of nitrodopamine was complete. This observation is in agreement with the unusually rapid cure time of PEG-ND (Figure 1).

Taken together, there are numerous differences and similarities between the oxidation cross-linking chemistry of nitrodopamine when compared to that of dopamine. For both adhesive moieties, the rate and extent of oxidative cross-linking increased with increasing pH. This is an indication that nitrodopamine oxidizes to its quinone form (Scheme 3 reaction 1)[44,45] prior to subsequent cross-link formation as the concentration of the deprotonated catechol increased when the pH approaches or exceeds its dissociation constant. The electron-withdrawing NO_2 group expands the number of

Scheme 3. Possible Reaction Pathways for PEG-ND[a]



[a]Nitrodopamine oxidizes to nitrodopamine quinone with the addition of $NaIO_4$ (**1**). Generation of an aryloxy radical leads to formation of nitrodopamine dimers and curing to the adhesive (**2**). Nitrodopamine quinone forms adduct with nucleophilic groups (e.g., -NH_2 found in lysine), resulting in interfacial bond formation with biological substrates (**3**). $\mathbf{R_1}$ and $\mathbf{R_2}$ represent PEG and proteins found on tissues, respectively.

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resonance structures and increases the stability of additional electrons in the ring, leading to a lowered pK_a. This lower pK_a likely increased the concentration of the oxidized species of nitrodopamine at a given pH when compared to that of dopamine and drastically enhanced the rate of cross-linking.

Nitrodopamine cross-linked mainly through dimerization of the catechol rings (Scheme 3, reaction **2**). The presence of a nitro-functional group is a steric hindrance and limited the formation of oligomers.[45] Recent reports also indicated that chemical modification of the catechol side chain modulates and reduces the degree of dopamine polymerization.[46−48] On the other hand, the unsubstituted catechols of dopamine and DOPA participate in different cross-linking pathways depending on the pH and are capable of forming oligomers through polymerization of the catechol groups.[12,21] This difference in the cross-linking pathways contributed to the difference in $NaIO_4$ concentration-dependent curing behavior between PEG-D and PEG-ND. Additionally, formation of the nitrodopamine dimer resulted in adhesive formulations with reduced cross-linking density and bulk mechanical properties. The oxidized forms of nitrodopamine and dopamine react with nucleophiles found on tissue substrates (i.e., $-NH_2$ and $-SH$ on lysine and cysteine, respectively) resulting in the formation of interfacial covalent bonds (Scheme 3, reaction 3). However, PEG-ND demonstrated significantly higher adhesive strength to tissue under mildly acidic conditions, as the oxidative cross-linking chemistry of nitrodopamine was minimally affected by the changes in pH as compared to that of dopamine. The lower pK_a of nitrodopamine likely contributed to a higher amount of oxidized species (i.e., semiquinone), which aided in its transition to quinone for reaction with amine group even if there is a higher amount of protonated $-NH_2$ on the surface of an acidic tissue. Reducing pK_a in the reactants have been found to increase the reactivity for Michael-type adduct formation.[49]

To our knowledge, this is the first report to demonstrate the bioadhesive properties of nitrodopamine. PEG-ND exhibited an extremely fast cure rate and its cross-linking chemistries (both cohesive and interfacial) were minimally hampered by the changes in pH. This is unlike PEG-D where there was a remarkable drop off in adhesive performance with a minor change in pH within the physiological range.[21] The use of nitrodopamine may be advantageous in designing adhesive biomaterials for repairing tissues that are more acidic (e.g., pH = 4−6 for skin,[50] pH = 6.7−7.1 for subcutaneous tissue,[41] pH = 7 for dysoxic tissue due to extensive hemorrhage[42]) or drug delivery vehicle needing to adhere to cancer tissues (pH ∼ 7).[43] Nitrodopamine is a natural occurring molecule, resulting from nitration of neurotransmitter, dopamine,[51,52] and is less toxic than other endogenous dopamine metabolites (e.g., 6-hydroxyldopamine).[53] In vitro cytocompatibility of nitrodopamine-modified polymers have also been favorable.[29,54] However, extensive biocompability testing will be required before incorporating nitrodopamine for future clinical applications. Finally, Shafiq et al.[29] demonstrated that PEG-ND is susceptible to light-mediated degradation. This unique feature provides an opportunity to use this biomimetic adhesive technology for designing a smart bioadhesive that can bond and debond on demand.

CONCLUSION

The effect of nitro-functionalization of dopamine on its cross-linking chemistry and bioadhesion was characterized. PEG-ND and PEG-D exhibited different dependence on the $NaIO_4$ concentration and pH, which affected their curing rate, mechanical properties, and adhesive performance differently. Nitro-functionalization reduced the pK_a of the catechol hydroxyl groups leading to extremely fast curing rate and higher reactivity toward nucleophiles found on tissue substrates even under mildly acidic pH. PEG-ND significantly outperformed PEG-D when adhering to biological substrates under acidic conditions. However, the presence of the nitro-group increased steric hindrance and prevented the formation of higher molecular weight oligomers. As such, dimerization of nitrodopamine resulted in the formation of PEG-ND network with reduced cross-linking density when compared to that of PEG-D. PEG-ND with reduced bulk cohesive properties exhibited lowed adhesive strength in neutral to basic conditions. The ability for nitrodopamine to rapidly cure and adhere to biological substrates in an acidic pH make it suitable for designing adhesive biomaterials targeted at tissues that are more acidic (i.e., subcutaneous, dysoxic, or tumor tissues).

ASSOCIATED CONTENT

Supporting Information

Additional information on the tensile properties of pericardium tissue, adhesive curing rate, molecular weight between cross-links, adhesion data, and UV−vis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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