Synthesis of 3,4-dihydroxyphenylalanine (DOPA) containing monomers and their co-polymerization with PEG-diacrylate to form hydrogels

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Abstract—L-3,4-Dihydroxyphenylalanine (DOPA) is an unusual amino acid found in mussel adhesive proteins (MAPs) that is believed to lend adhesive characteristics to these proteins. Most previous efforts to incorporate DOPA into hydrogels have utilized oxidative cross-linking, which is hypothesized to reduce the adhesive properties of DOPA and requires reagents that are harmful to biological tissues. In this paper, we describe the synthesis of N-methacrylated DOPA monomers and their copolymerization with poly(ethylene glycol) diacrylate (PEG-DA) using either ultraviolet (UV) or visible light. The effect of DOPA containing monomers on gelation time, gel conversion and elastic modulus of the photocured hydrogels was investigated. Despite a retarding effect of DOPA on photopolymerization, DOPA was successfully incorporated into hydrogels with elastic moduli suitable for many biomedical applications. The incorporation of DOPA into hydrogels by photopolymerization may lead to new adhesive hydrogels for medical applications.

Key words: DOPA; poly(ethylene glycol); hydrogel; bioadhesive; photopolymerization; DMPA; contact mechanics.

INTRODUCTION

Bioadhesive hydrogels have many potential applications in medicine and dentistry, such as surgical adhesives and as vehicles for drug delivery to mucosal surfaces [1]. Clinically successful adhesive biomaterials should have the following primary attributes: (1) the ability to rapidly solidify or polymerize in situ from a fluid

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precursor; (2) the ability to form strong and durable bonds to tissue surfaces even in the biological environment; and (3) biocompatibility. However, the current generation of medical adhesives seldom meet all of these requirements due to lack of adhesion strength, possibility of viral contamination, poor adhesion in the wet environment and toxicity concerns [2]. Therefore, there exists a need for the development of new, safe and rapidly curable bioadhesives.

Successful water resistant adhesives have been found in marine organisms including mussels, barnacles, kelps and oysters, whose survival is dependent upon their ability to attach to wet surfaces [3]. Of particular interest are the mussel adhesive proteins (MAPs) secreted by marine mussel species such as Mytilus edulis. MAPs are remarkable underwater adhesive materials initially secreted as proteinaceous fluids that harden in situ to form an adhesive plaque that anchors marine and freshwater mussels to the substrates upon which they reside [3, 4]. One of the unique structural features of MAPs is the presence of L-3,4-dihydroxyphenyalanine (DOPA), an amino acid which is believed to be responsible for the adhesive characteristics of MAPs. Although the adhesive mechanisms of MAPs are not fully understood, oxidation of DOPA is believed to lead to intermolecular cross-link formation that results in the curing of the plaques [5].

In an effort to take advantage of the adhesive properties of DOPA, several groups have reported the synthesis and characterization of DOPA-containing peptides and polymers [4–13]. However, the curing of these DOPA-containing compounds was typically accomplished by oxidation of DOPA residues to form DOPA-quinone, which participated in intra- and/or intermolecular cross-linking reactions to form a gel network. The oxidized forms of DOPA that result from such reactions are believed to be less adhesive than unoxidized DOPA [5]. DOPA-containing proteins and polymers exhibit better adhesion to both metallic and mucosal surfaces when DOPA residues are not oxidized [11, 14]. Furthermore, complete characterization of the DOPA adhesion mechanism, as well as future in vivo applications, are complicated by the presence of oxidation reagents necessary for curing. Thus, we have been searching for alternative methods of hydrogel formation that can preserve the unoxidized adhesive form of DOPA [15].

The goal of this work was to incorporate DOPA into a gel network by photopolymerization. Synthesis of N-methacrylated DOPA monomers is described and these monomers were copolymerized with poly(ethylene glycol) diacrylate (PEG-DA) to form hydrogels by photopolymerization. Gel conversion and DOPA incorporation as a function of DOPA monomer concentration were determined for gels polymerized by ultraviolet and visible light initiating systems. Finally, a contact mechanical test was performed on the photocured gels and the elastic modulus of these gels was obtained by fitting the load-displacement data using a Hertzian relationship [16].
MATERIALS AND METHODS

Materials

PEG (\(M_w\) 8000), pentafluorophenol, 1,3-dicyclohexylcarbodiimide (DCC), 4,7,10-trioxa-1,13-tridecanediamine, fluorescein sodium salt (FNa\(_2\)) and ascorbic acid (AA) were purchased from Sigma (St. Louis, MO, USA). L-DOPA, sodium borate, methacryloyl chloride, \(t\)-butyldimethylsilyl chloride (TBDMS-Cl), di-\(t\)-butyl dicarbonate, methylcrylic anhydride, sodium molybdate dihydrate, sodium nitrite, 2,2’-dimethoxy-2-phenyl-acetonephenone (DMPA), acryloyl chloride, 1,8-diazabiclo[5.4.0]undec-7-ene (DBU), tetrabutylammonium fluoride (TBAF), 4-(dimethylamino)-benzoic acid (DMAB) and 1-vinyl-2-pyrrolidone (VP) were purchased from Aldrich (Milwaukee, WI, USA). Camphorquinone (CQ) was obtained from Polysciences (Warrington, PA, USA). Titanium oxide (TiO\(_2\)) surfaces were prepared by electron beam physical evaporation onto silicon (Si) wafer and cleaned in plasma chamber prior to testing. Unless otherwise noted, all chemical reagents were used as received.

Synthesis of N-methacryloyl 3,4-dihydroxy-L-phenylalanine (1)

1.15 g (5.69 mmol) of Na\(_2\)B\(_4\)O\(_7\) was dissolved in 30 ml of water. The solution was degassed with Ar for 30 min, after which 0.592 g (3.0 mmol) of L-DOPA was added and stirred for 15 min. 0.317 g (3.0 mmol) of Na\(_2\)CO\(_3\) was then added, the solution was cooled to 0°C and 0.3 ml (3.0 mmol) of methacryloyl chloride was slowly added with stirring. The pH of the solution was maintained above 9 with Na\(_2\)CO\(_3\) during the reaction. After stirring for 1 h at room temperature, the solution was acidified to pH 2 with concentrated HCl. The mixture was extracted with ethyl acetate three times. After washing with 0.1 M HCl and drying over anhydrous MgSO\(_4\), the solvent was removed in vacuo to yield crude light brown solid. The product was further purified by elution from a silica gel column with dichloromethane (DCM) and methanol (95:5). After evaporating the solvent, a white, sticky solid was obtained with a product yield of 35%. \(^1\)H-NMR (500 MHz, acetone-\(d_6\)):
\[
\begin{align*}
\delta &\quad 7.1 \; d \; (1 \; H, \; -NH-); \\
6.6–6.8 &\quad (3 \; H, \; C_6H_3(OH)_2-); \\
5.68 &\quad s \; (1 \; H, \; CHH=); \\
5.632 &\quad s \; (unknown \; peak); \\
5.33 &\quad s \; (1 \; H, \; CHH=); \\
4.67 &\quad m \; (1 \; H, \; -CH-); \\
2.93–3.1 &\quad m \; (2 \; H, \; CHH=); \\
1.877 &\quad s \; (3 \; H, \; -CH_3).
\end{align*}
\]

Synthesis of 3,4-bis(\(t\)-butyldimethylsilyloxy)-L-phenylalanine (2)

Compound 2 was prepared by modification of a literature procedure [17]. 3.60 g (24.0 mmol) of TBDMS-Cl was dissolved in 18 ml of anhydrous acetonitrile. 1.60 g (8.0 mmol) of L-DOPA was added to the solution, the suspension was stirred and cooled to 0°C and 3.6 ml of DBU (24.0 mmol) was added. The reaction mixture was then stirred for 24 h at room temperature. Addition of cold acetonitrile to the reaction solution resulted in a colorless precipitate. The precipitate was filtered and washed with cold acetonitrile several times followed by drying in vacuum. White
powder was obtained with a yield of 78%. $^1$H-NMR (500 MHz, methanol-d): $\delta$ 6.7–6.9 (3 H, C$_6$H$_3$(O–Si–)$_2$–); 3.72 (m, 1 H, –CH–); 2.82–3.2 (m, 2 H, –CH$_2$–); 1.0 (d, 18 H, –C(CH$_3$)$_3$); 0.2 (d, 12 H, Si–CH$_3$).

Synthesis of 3,4-bis(t-butyldimethylsilyloxy)-N-t-butyloxycarbonyl-L-phenylalanine (3)

Compound 3 was prepared by modification of a literature procedure [17]. 1.60 g (3.77 mmol) of 2 was added to 10 ml of water containing 0.34 g (4.05 mmol) of NaHCO$_3$, 0.96 g (4.30 mmol) of di-t-butyldicarbonate in 10 ml of tetrahydrofuran was added and the reaction mixture was stirred for 24 h at room temperature. After evaporation of tetrahydrofuran, 10 ml of water was added to the residue. The solution was acidified with dilute HCl to pH 5 and extracted three times with ethyl acetate. After drying over anhydrous MgSO$_4$, the solvent was removed in vacuo. The crude product was purified by column chromatography (silica gel; eluent, 10% methanol in DCM). A white solid was obtained with a yield of 70% after evaporating the eluding solvent. $^1$H-NMR (500 MHz, methanol-d): $\delta$ 6.68–6.81 (3 H, C$_6$H$_3$(O–Si–)$_2$–); 4.28 (m, 1 H, –CH–); 2.78–3.08 (m, 2 H, –CH$_2$–); 1.4 (s, 9 H, –O–C(CH$_3$)$_3$); 1.0 (d, 18 H, –Si–C(CH$_3$)$_3$); 0.2 (d, 12 H, Si–(CH$_3$)$_2$).

Synthesis of 3,4-bis(t-butyldimethylsilyloxy)-N-t-butyloxycarbonyl-L-phenylalanine pentafluorophenyl ester (4)

1 g (1.90 mmol) of 3 and 0.351 g (1.90 mmol) of pentafluorophenol were dissolved in a solvent mixture of 24 ml of dioxane and 1 ml of DMF and 0.432 g (2.10 mmol) of DCC was added at 0°C. The solution was stirred for 1 h at 0°C and for 1 h at room temperature, after which the solution was filtered to remove dicyclohexylurea and evaporated in vacuo. The product 4 was purified by column chromatography (silica gel; eluent, hexane/ethyl acetate 11 : 2). After removing the eluent, a pure white, sticky solid was obtained with a yield of 66%. $^1$H–NMR (500 MHz, CDCl$_3$): $\delta$ 6.65–6.81 (3 H, C$_6$H$_3$(O–Si–)$_2$–); 4.85 (m, 1 H, –CH–); 3.05–3.2 (m, 2 H, –CH$_2$–); 1.41 (s, 9 H, –O–C(CH$_3$)$_3$); 1.0 (d, 18 H, –Si–C(CH$_3$)$_3$); 0.2 (d, 12 H, –Si–(CH$_3$)$_2$).

Synthesis of N-(13′-amino-4′,7′,10′-trioxatridecanyl)-t-butyloxycarbonyl-3′,4′-bis(t-butyldimethylsilyloxy)-L-phenylalaninamide (5)

0.869 g (1.26 mmol) of 4 in 10 ml of DCM was added drop wise to a mixture of 2.07 ml (9.44 mmol) of 4,7,10-trioxa-1,13-tridecanediamine and 1.32 ml (9.44 mmol) of Et$_3$N in 1 ml DMF over 30 min at 0°C. The solution was stirred at room temperature for another 2 h and then the solvent was removed under vacuum. The crude product was loaded onto silica gel and eluted with DCM, 5% methanol in DCM, 10% methanol in DCM and 15% methanol in DCM. The solvent was removed under vacuum to yield 5 as a white solid. The yield was 63%.
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1H-NMR (500 MHz, acetone-\textit{d}_6): \(\delta\ 7.38\ (m, 1\ H, -CONH\ ->); 6.60–6.80\ (3\ H, C_6H_5(O-Si-\textit{d}_2\-); 5.26\ (m, 1\ H, -CONH\ ->); 4.30\ (m, 1\ H, -CH\ ->); 3.4–3.8\ (m, 12\ H, -CH_2O\-); 3.03–3.4\ (m, 4\ H, -CH_2-NH\-, -CH_2-NH\_2\); 2.78–3.02\ (m, 2\ H, -CH_2\-); 2.0\ (m, 2\ H, -CH_2\-); 1.7\ (m, 2\ H, -CH_2\-); 1.39\ (s, 9\ H, -O-C(CH_3)_3\); 1.0\ (d, 18\ H, -Si-C(CH_3)_3\); 0.2\ (d, 12\ H, -Si-(CH_3)_2\).

Synthesis of N-(13-(\textit{N}°-t-butyloxycarbonyl-L-3°,4°-bis(t-butyldimethylsilyloxy)-4,7-10-trioxtatridecanyl)-methacrylamide (6)

0.572\ g\ (0.79\ mmol)\ of 5 and 0.166 ml\ (1.18\ mmol)\ of Et_3N were dissolved in 5 ml of anhydrous chloroform, to which 0.176 ml\ (1.18\ mmol) of methacrylic anhydride was added. The solution was stirred at room temperature for 3\ h, then solvent was removed \textit{in vacuo}. Pure 6 was obtained by column chromatography (silica gel; eluent, ethyl acetate) as a white, sticky solid with a yield of 61%. 1H-NMR (500 MHz, CDCl_3): \(\delta\ 6.60–6.80\ (3\ H, C_6H_3(O-Si\-)); 6.40\ (m, 1\ H, -CONH\-); 5.71\ s\ (1\ H, CHH\=); 5.30\ s\ (1\ H, CHH\=); 5.096\ (m, 1\ H, -CONH\-); 4.21\ (m, 1\ H, -CH\-); 3.2–3.65\ (m, 16\ H, -CH_2O, -CH_2-NH\-, -CH_2-NH\_2\); 2.80–2.99\ (m, 2\ H, -CH_2\-); 1.96\ (s, 3\ H, -CH_3\); 1.81\ (m, 2\ H, -CH_2\-); 1.68\ (m, 2\ H, -CH_2\-); 1.40\ (s, 9\ H, -O-C(CH_3)_3\); 1.0\ (d, 18\ H, -Si-C(CH_3)_3\); 0.2\ (d, 12\ H, -Si-(CH_3)_2\).

Synthesis of N-(13-(\textit{N}°-t-Boc-L-3°,4°-dihydroxylphenylalaninamido)-4,7-10-trioxtatridecanyl)-methacrylamide (7)

To a 10 ml round-bottom flask were added 0.344\ g\ (0.433\ mmol)\ of 6, 3 ml of THF and 0.137 g\ (0.433\ mmol) of TBAF. The solution was stirred at room temperature for 5\ min, then 3\ ml of 0.1 M HCl was added. The solution was extracted three times with DCM, after which the solvent was evaporated in \textit{vacuo}. 7 was obtained as a white solid by column chromatography (silica gel; eluent, 7% methanol in DCM) with a yield of 63%. 1H-NMR (500 MHz, acetone-\textit{d}_6): \(\delta\ 7.90\ (m, 1\ H, -CONH\-); 7.23–7.40\ (d, 2\ H, C_6H_2(OH)_2\-); 6.56–6.76\ (3\ H, C_6H_2(OH)_2\-); 5.930\ (m, 1\ H, -CONH\-); 5.71\ (s, 1\ H, CHH\=); 5.30\ s\ (1\ H, CHH\=); 4.20\ (m,1\ H, -CH\-); 3.1–3.60\ (m, 16\ H, -CH_2O, -CH_2-NH\-, -CH_2-NH\_2\); 2.70–2.95\ (m, 2\ H, -CH_2\-); 1.93\ s\ (3\ H, -CH_3\); 1.78\ (m, 2\ H, -CH_2\-); 1.65\ (m, 2\ H, -CH_2\-); 1.39\ (s, 9\ H, -O-C(CH_3)_3\).

Synthesis of PEG-diacrylate (PEG-DA)

Acrylation of PEG was performed using a similar method as described by Hern and Hubbell [18]. 40\ g\ (5\ mmol) of PEG was dried by azeotropic evaporation in benzene and then dissolved in 150 ml of DCM. 4.18\ ml\ (30\ mmol) of Et_3N and 3.6\ ml\ (40\ mmol) of acryloyl chloride (Aldrich) was added to the polymer solution. The mixture was refluxed with stirring for 5\ h and allowed to cool at room temperature overnight. Ether was added to the mixture to form a faint yellow precipitate. The
crude product was then dissolved in saturated NaCl solution, which was heated to 60°C to form two layers. DCM was added to the top layer and MgSO₄ was added to remove moisture. After filtration of MgSO₄, the volume of the solvent was reduced in vacuum and the sample was precipitated in ether. The final product was dried in vacuum and stored at −15°C. The yield was 75%. ¹H-NMR (500 MHz, D₂O): δ 6.47 (d, 1 H, CH=CH); 6.23 (m, 1 H, C≡CH→C(=O)→O→); 6.02 (d, 1 H, CH=CH→C→); 4.35 (m, 2 H, −CH₂−O−C(=O)→C≡C); 3.23–3.86 (PEG CH₂).

Photopolymerization

Precursor solutions of PEG-DA, 1, 7 and photoinitiator were prepared and mixed immediately before photopolymerization. Stock solutions of PEG-DA (200 mg/ml) and 1 (40 mg/ml) were dissolved in N₂-purged phosphate buffered saline (PBS, pH 7.4), whereas 7 (60 mg/ml) was dissolved in 50:50 PBS/95% ethanol previously purged with N₂. To prepare the final polymerization mixture, solutions of 1 or 7 were combined with PEG-DA to achieve a final concentration of PEG-DA and DOPA derivatives of 150 mg/ml. 100 µl of the mixture was then added to a disc-shaped mold (100 µl, diameter 9 mm, depth 2.3 mm, Secure Seal® SA8R-2.0, Grace Bio-Lab, OR, USA) and irradiated for up to 20 min either with an UV lamp (Black Ray® Lamp, 365 nm, model UVL-56, UVP, Upland, CA, USA) or a blue light lamp (VIP®, 400–500 nm, Bisco, Schaumburg, IL, USA). For UV-initiated photocuring, DMPA (600 mg/ml in VP) was added to the polymeric solution to make a final concentration of 34 mM. Visible light-induced curing was performed using either CQ (100 mg/ml in VP, final concentration 150 mM) with DMAB (30 mg/ml in VP, final concentration 151 mM), or FNa₂ (188 mg/ml in PBS, final concentration 2 mM) with AA (100 mg/ml in PBS, final concentration 17 mM) as the photoinitiator. The final VP concentration was adjusted to be between 135 and 300 mM.

After irradiation, the gels were blotted with filter paper to remove the liquid surface layer and weighed. Percent gel conversion was then determined by dividing the weight of the gel by the weight of 100 µl of the precursor solution.

Determining DOPA incorporation

The amount of DOPA incorporated into the photopolymerized gel was determined using a modification of the colorimetric DOPA assay developed by Waite and Benetict [19]. Photo-cross-linked gels were stirred in 3 ml of 0.5 M HCl to extract DOPA monomers that were not incorporated into the gel network. 0.9 ml of the nitrite reagent (1.45 M sodium nitrite and 0.41 M sodium molybdate dihydrate) and 1.2 ml of 1 M NaOH were added to 0.9 ml of the extraction solution and the absorbance (500 nm) of the mixture was recorded using a Hitachi U-2010 UV-Vis spectrophotometer within 2 to 4 min of NaOH addition. Standard curves were constructed using known 1 and 7 concentrations.
Mechanical test

Hydrogels were formed in the shape of a hemisphere by loading 25 µl of the polymer mixture onto a glass slide treated with 1 H, 1 H, 2 H, 2 H-perfluorooctyltrichlorosilane. Gels were irradiated for 10 min, dialyzed in 0.15 M HCl for at least 24 h to extract unincorporated DOPA monomers and then equilibrated in PBS for greater than 15 min prior to testing. Figure 1 shows a detailed schematic diagram of the testing apparatus employed to characterize the mechanical properties of these gels. To determine the gel modulus, hemispherical gel caps were attached to one end of a steel cylinder (diameter 6 mm, length 30 mm) using superglue. The other end of the cylinder was attached to a piezoelectric stepping motor (IW-701-00, Burleigh Instruments, Fishers, NY, USA) aligned in series with a 50 g load transducer (FTD-G-50, Schaevitz Sensors, Hampton, VA, USA) with a resolution of approx. 0.1 mN. A fiber optic displacement sensor (RC100-GM2OV, Philtec, Annapolis, MD, USA) measured the axial movement of the steel rod. A TiO$_2$-coated Si wafer was positioned below the hydrogel and the TiO$_2$ surface was flooded with PBS in order to maintain the hydration of the gel. The indenter was advanced at 5 µm/s until a maximum compressive load of 4 mN was measured.

Elastic moduli were calculated by assuming Hertzian mechanics [16] for the specific case of non-adhesive contact between an incompressible elastic hemisphere and a rigid plane, in which case the Hertzian relationship between load ($P_h$) and

![Figure 1. Testing apparatus for characterizing the mechanical properties of polymer hydrogels.](image-url)
displacement ($\delta_h$) becomes [20]:

$$P_h = \frac{16R^{1/2}E}{9}\delta_h^{3/2},$$

(1)

where $R$ and $E$ are the radius of curvature and the elastic modulus of the hemispherical gel, respectively. The radius of curvature of the gels was determined from height and width measurements obtained from a photograph of the gel.

**Statistical analysis**

Statistical analysis of percent gel conversion, extent of DOPA incorporation and gel modulus was performed using analysis of variance (ANOVA) and Tukey post-hoc analysis using a significance level of $P = 0.05$.

**RESULTS AND DISCUSSION**

Novel photocurable DOPA-containing monomers were synthesized and copolymerized with poly(ethylene glycol) diacrylate (PEG-DA) to form DOPA-containing hydrogels. The new monomers 1 and 7 combine an adhesive moiety, DOPA and a polymerizable methacrylate group with or without an oligomeric ethylene oxide linker. To our knowledge, this is the first report of DOPA-containing hydrogels formed by photopolymerization. PEG was chosen as the structural gel-forming polymer because of its favorable properties of biocompatibility, non-toxicity and non-immunogenicity [21, 22]. Furthermore, photocuring of PEG-DA macromonomers to form biocompatible hydrogels have been demonstrated by numerous investigators [18, 23, 24]. Copolymerization of 1 and 7 with PEG-DA was hypothesized to be a convenient method to enhance the adhesive properties of PEG hydrogels, which are generally considered to be poorly adhesive.

**Synthesis of polymerizable DOPA monomers 1 and 7**

Preparation of $N$-methacryloyl amino acids using methacryloyl chloride has been previously reported by Nishiyama et al. [25–27]. However, existing methods were not employed in this study because of the reactivity of the catechol side chain of DOPA toward methacryloyl chloride, as well as the known susceptibility of catechols to oxidation under alkaline reaction conditions. Instead, for synthesis of monomer 1 DOPA was first protected by dissolving in borate buffer at pH 9, forming a DOPA-borate complex stable at pH between 7 and 10 [19, 28] that would function to protect the catechol during reaction. As shown in Scheme 1, 1 was then prepared by adding methacryloyl chloride to the borate-protected DOPA, after which the borate-catechol complex was easily disrupted through acidification of the solution [19, 28].

Monomer 7 was synthesized with an oligomeric oxyethylene spacer between DOPA and methacrylate moieties, to enhance aqueous solubility of the monomer, as
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Scheme 1. Synthesis of 1.

Scheme 2. Synthesis of 7.

well as to facilitate the interaction of the pendant catechol with substrate surfaces. As shown in Scheme 2, in this case DOPA was protected with TBDMS and Boc groups, followed by activation of the DOPA carboxyl group with pentafluorophenol. The subsequent DOPA derivative was reacted with an excess of oxyethylene oligomer, 4,7,10-trioxa-1,13-tridecanediamine, after which the free amine terminus was reacted with methacryloyl chloride. TBAF, a mild deprotecting reagent [29], was then used to remove TBDMS.

Photopolymerization

The initiating systems used in this study (DMPA, CQ/DMAB, AA/FNa₂) have all been previously investigated for radical photopolymerization. Several investigators have used DMPA in the formulation of hydrogels from acrylated PEG macromers [18, 23, 24]. CQ in conjunction with an amine has been used exten-
Figure 2. Percent gel conversion of PEG-DA (150 mg/ml) as a function of length of UV exposure.

Figure 3. Percent gel conversion of DOPA-containing gels after 10 min UV exposure as function of mol% 1 or 7 in the precursor solution. The filled circles (●) and solid regression lines denote gels containing 1, while open circles (○) and dashed regression lines denote gels containing 7.
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Scheme 3. Hydrogel formation via copolymerization of 1 or 7 with PEG-DA.

sively in the curing of dental resins [30, 31] and the usage of FNa₂ with a reducing agent for visible light photoinitiation have also been studied for biomedical purposes [32, 33].

After 2 min of UV irradiation, PEG-DA solutions containing DMPA as the initiator formed clear, colorless gels covered by a liquid surface layer, which was believed to be due to the inhibiting effect of molecular oxygen [34]. As shown in Fig. 2, gel conversion reached more than 75 wt% after 2 min of UV irradiation and increased to greater than 85 wt% upon irradiation for more than 5 min. Gelation of PEG-DA also occurred in 4 min or less when visible light initiators were used (4 min for CQ/DMAB and 3 min for FNa₂/AA).

Copolymerization of PEG-DA with 1 or 7 is illustrated in Scheme 3. Polymerization of these mixtures was qualitatively similar to pure PEG-DA, although addition of 1 or 7 to the PEG-DA precursor solution resulted in a decrease in gel conversion that was dependent on DOPA monomer concentration and initiating system. For example, in DMPA-initiated UV polymerization (Fig. 3), gel conversion was reduced to less than 85 wt% in the presence of 2.5 mol% or more 1 and 7. However, the extent of gel conversion was not statistically different between 1 and 7 gels. Similar DOPA concentration-dependent inhibition was observed for the visible-light-induced initiators. For FNa₂/AA- and CQ/DMAB-initiated mixtures, addition of
Figure 4. Mol fraction of DOPA incorporated into the gel network as a function of mol% 1 or 7 in the precursor solution. Symbols as in Fig. 2.

33.3 mol% of 1 increased the gelation time to more than 8 min. Nevertheless, solutions containing 1 and 7 were still capable of photocuring even at a relatively high mol% of DOPA.

The composition and elastic modulus of UV polymerized gels (DMPA initiator) were further analyzed in detail. After photocuring, DOPA-containing gels were dialyzed in 0.5 M HCl to extract unreacted DOPA monomer. To quantify the extent of DOPA incorporation, the dialysate was assayed using the DOPA colorimetric assay of Waite and Benedict [19] and the results used to calculate the amount of DOPA incorporated into the gel network. Figure 4 shows the mole fraction of DOPA incorporated into the gel network, as a function of mol% monomer 1 and 7 in the precursor solution. There was no significant difference in the mole fraction DOPA incorporated between samples containing 1 and 7.

Direct evidence for the presence of DOPA in the gels was obtained by immersing the intact dialyzed hydrogels in nitrite reagent followed by NaOH [19]. The initially colorless gels turned bright yellow after the addition of the nitrite reagent and then red following the addition of excess base (Fig. 5). This color transition is typical of catechols [19], indicating that the unoxidized form of DOPA was incorporated into the hydrogels through photopolymerization. The intensity of the red color also reflected the concentration of DOPA incorporated into the photocured gels, as seen
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Figure 5. Photographs of UV-photocured PEG gels containing (A) 0, (B) 2.6, (C) 14.3 and (D) 33.3 mol% of 1 after dialysis with 0.5 M HCl and treatment with DOPA assay reagents.

in Fig. 5. PEG-DA gels that did not contain DOPA (Fig. 5A) remained colorless with the addition of nitrite reagent and NaOH solution.

Mechanical properties of DOPA-containing hydrogels

Load versus displacement data, obtained by indentation of hydrogel hemispheres onto a rigid flat surface, were recorded for selected gels to determine the elastic modulus. All of the loading data obtained from these experiments were fitted well ($R^2 > 0.99$) by Hertzian curves (equation (1)). Equation (1) is valid when the average strain in the deformed region of the gel is small. For our geometry, the average strain can be approximated by $(\delta/R)^{1/2}$, which is indeed very small in our experiment. An example of a typical Hertzian fit is shown in Fig. 6 for a PEG-DA hydrogel. The non-linear relationship between $P_h$ and $\delta_h$ is not an indication of a non-linear constitutive model for the material, but is instead due to the changing geometry as $\delta_h$ is increased. The load increases non-linearly with displacement because the contact area between the gel and the substrate increases with displacement. The curve-fitting procedure allowed elastic moduli to be calculated based on the proportionality factor of the curve fit.

Table 1 shows the average DOPA content in each gel type along with the mean values of $E$ calculated by the methods described above. Addition of 33 mol% of monomers 1 and 7 into the precursor solutions lowered the average Young’s modulus values of the photocured gel by 34% and 29%, respectively. The average Young’s modulus values obtained for gels containing 1 and 7 were not statistically different. Despite the decrease in modulus compared to PEG-DA gels, DOPA-containing gels still exhibited moduli suitable for many biomedical applications.

While the presence of DOPA-containing monomers did not prevent polymerization, longer irradiation time was needed to achieve gelation when monomers 1 and 7 were present in the precursor solution. Gel conversion, extent of DOPA incorporation and elastic modulus also decreased with increasing DOPA concentration. These observations suggest that the DOPA-containing monomers exert a retarding effect on free radical polymerization. Although no literature references
to inhibitory or retarding effects of catecholic compounds were found, related phenolic compounds such as hydroquinone and 4-methoxyphenol are known inhibitors of radical polymerization, acting to quench excited initiators and retard the initiation process [35–37]. In the presence of oxygen, these inhibitors can also interact with peroxy radicals and terminate the propagating kinetic chain [38, 39]. Furthermore, melanin, a DOPA-containing polyphenol, has been found to act as a radical scavenger [40]. The presence of a liquid-like layer at the surface of the gels is consistent with an enhanced inhibition due to atmospheric oxygen and with the fact that the gels did not measurably adhere to the oxide substrates used for the mechanical
measurements. Elucidation of the exact inhibition/retardation mechanism and its influence on the adhesive response of the gels will require further study.

In summary, N-methacrylated DOPA monomers were synthesized and copolymerized with PEG diacrylate to form hydrogels. Despite a retarding inhibitory effect of DOPA on photopolymerization, DOPA-containing monomers were successfully incorporated into PEG hydrogels. To our knowledge, this is the first report of a DOPA-containing hydrogel formed by photopolymerization. The resulting hydrogels do not require oxidizing reagents to gel and possess moduli sufficient for use in many biomedical applications. The incorporation of DOPA into hydrogels in the unoxidized state may prove to be an important tool in understanding the role of DOPA in mussel adhesive proteins and may lead to new adhesive hydrogels for biomedical applications. Experiments aimed at determining the adhesive properties of these hydrogels are underway.

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