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Surgical sealants with tunable swelling, burst pressures, and biodegradation rates

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Abstract: We developed two types of polyethylene glycol (PEG)-based surgical sealants, which we have termed the PER and PRO series. In one, the PRO series, an 8-arm PEG containing activated carbonyl end-groups was reacted with a 4-armed amino-PEG. In the second, the PER series, a 4-arm PEG containing bi-functional end groups with four azides and four activated esters was reacted by strain-promoted alkyne-azide cycloaddition with a 4-arm cyclooctyne-PEG to give a near-ideal Tetra-PEG hydrogel. The sealants showed predictably tunable strength, swelling, adhesion, and gelation properties. The gels were compared to commercially available PEG-based sealants and exhibit physical properties equivalent to or better than the standards. Variants of each gel-format were prepared that contained a β-eliminative cleavable linker in the crosslinks to control degradation rate. Linkers of this type self-cleave with half-lives spanning from hours to years, and offer the unique ability to precisely tune the degradation to match the healing process. In addition, these linkers could serve as cleavable tethers for controlled drug release. © 2016 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 00B: 000–000, 2016.

Key Words: tetra-PEG hydrogels, cleavable linkers, surgical sealants, hydrogel, controlled degradation

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INTRODUCTION

Surgical sealants are natural or synthetic polymers used in surgery to prevent liquid and air leaks.[1,2] They are generally composed of three functional units: (i) a sealant or impenetrable gel, (ii) an adhesive that serves to bind the sealant to tissue components, and (iii) cleavable cross-linkers to facilitate biodegradation. The sealant is usually spread or sprayed as a liquid onto the target tissue shortly after surgery, and rapidly forms a fluid- or air-sealing gel that adheres to tissues. The requirements for surgical sealants are: (a) rapid *in situ* polymerization from liquid components, (b) strong bonding to tissue, (c) appropriate swelling and strength for the application site, and (d) biodegradation at an appropriate rate.

One major class of synthetic sealants approved for clinical use employ polyethylene glycol (PEG) polymers. These include Coseal® (suture sealant), Duraseal® and Duraseal Xact® (spinal sealants), and Progel® (pleural sealant). In addition to use as surgical sealants, some of these polymers are also used as anti-adhesion barriers: for example, Duraseal Xact as Sprayshield®, and Coseal as Adhibit®; and recently, the components of Duraseal have been approved by the U.S. FDA as Resure Sealant® for sealing corneal incisions after cataract surgery. Many similar or identical PEG polymers are used as barriers to prevent tissue adhesion.[1]

The common polymerization chemistry theme for all of these PEG-based polymers is that one component is a multiarm PEG containing reactive *N*-hydroxysuccinimidyl esters (HSE) or *N*-hydroxysuccinimidyl carbonates (HSC) end groups. This component is rapidly mixed with a second polymer component containing multiple amine or thiol nucleophiles (e.g., tetra-thiol PEG in Coseal, tri-lysine in Duraseal and Resure, albumin in Progel) via a dual barrel syringe and directly applied to the target tissue. The HSE or HSC end groups react with the multifunctional nucleophile to form the polymeric sealant, and some react with Lys amino groups of tissue proteins (e.g., collagen) to form tissue adhesive bonds. In all cases, the reactions result in a hydrogel polymer affixed to tissue by amide or carbamate linkages

The marketed PEG-based sealants also contain hydrolyzable ester groups in the crosslinks to cause biodegradation. The biodegradation rate is governed by the susceptibility of such groups to hydrolysis, as well as the number of cleavable crosslinks per monomeric unit in the polymer. The *in vivo* hydrolysis rates are further modified by buffer catalysis and

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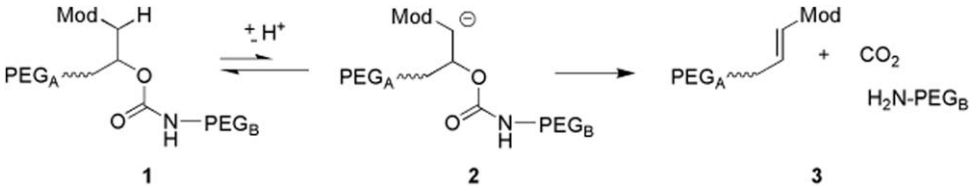


SCHEME 1. The mechanism of beta-elimination linker cleavage.

esterases making it difficult to adjust the biodegradation rates or to predict them from *in vitro* data. As such, biodegradation rates of these gels are usually empirically determined.

We have recently described polymer crosslinkers that undergo non-hydrolytic β-eliminative cleavage and lead to predictable gel degradation times.[3,4] Here (Scheme 1), polymer crosslinks are formed by a cleavable carbamate (**1**); the β-carbon of the carbamate has an acidic carbon-hydrogen bond (C—H) and also contains an electron-withdrawing pK_a "modulator" (Mod) that controls the acidity of that C—H bond. On proton loss (**2**), a rapid β-elimination occurs to cleave the linker-carbamate bond (**3**). The rate of linker cleavage is proportional to the acidity of the proton, which is controlled by the electron withdrawing effect of the modulator, and is first order with respect to hydroxide ion. Hence, by incorporating such crosslinking agents into PEG-based sealants, we can optimize the rate of biodegradation so it is appropriate for the intended use of the sealant.

Each tissue and application environment presents unique demands on sealants.[5] Vessels or organs with higher pressures or mobility require biomaterials with high bursting strength that are soft, deformable and elastic. Tolerance for post-application swelling may likewise differ depending on use; for example, whereas vascular sealants may be tolerant of swelling, dural sealants require minimal swelling to avoid neural compression. Lastly, the residence time of a sealant must be long enough to serve its purpose, but not so long that it interferes with or modifies the normal healing process. Clearly, it would be ideal if a single surgical sealant could be tuned to have optimal properties for the tissue and purpose it is intended for—notably, gelation time, swelling, adhesion, elastic modulus, burst pressure, and degradation rate. This might not be practical *per se*, but it would not be unreasonable to have several variations of a format so the best available for a specific purpose could be chosen.

In this work, we prepared and studied two types of sealants. In one, the PRO series, an 8-arm PEG containing HSE or HSC end groups was reacted with a 4-armed amino PEG. In the second, the PER series, a 4-arm PEG containing bifunctional end groups with exactly 4 azides and 4 HSEs was reacted by strain-promoted alkyne-azide cycloadditions (SPAAC) with a 4-arm PEG dibenzocyclooctyne (DBCO) to give a near-ideal Tetra-PEG hydrogel.[6] Related Tetra-PEG gels are known to have a homogeneous network and extraordinary strength and deformability.[7,8] All of the gels contained unreacted HSE or HSC groups for tissue adhesion. Although many of the gels studied contained stable crosslinks, variants of each were also prepared that contained a β-eliminative cleavable linker in the crosslinks to control the degradation rate. Previous work has described a large number of such linkers with degradation rates spanning a few days to over a year.[3,6]

MATERIALS AND METHODS

Nomenclature

The two hydrogel precursors are referred to as Prepolymer A and B, the components of which are described below.

The nomenclature devised to specify gel formulations (Figure 1) consists of the polymer series (PRO or PER) followed by L if the gel contains cleavable crosslinks; a series of hyphenated numbers describe (A) the molecular weight in kDa of Prepolymer A, (B) the molecular weight in kDa of Prepolymer B, (C) the ratio of end groups of Prepolymer A and B—[HSE]/[amine] end groups for the PRO series or [azide]/[DBCO] end groups for the PER series— and D) the initial concentration of the polymer % (w/v) as mixed. For example PRO-L-A-B-C-D refers to a PRO series gel containing cleavable crosslinkers and composed of Prepolymer A with MW A kDa, Prepolymer B with MW B, [HSE]/[amine] ratio of C, and the initial % polymer (w/v) D.

SYNTHESIS

PRO and PRO-L series

The PRO sealant gels (Scheme 2) were prepared by polymerizing a 20- or 40-kDa 8-arm PEG having 8-HSE end groups (Prepolymer A) with 2-kDa tetra-amino PEG (Prepolymer B). The PRO-L sealants (Scheme 3) used a 20-kDa PEG with 8-HSC end groups connected by a cleavable linker (Prepolymer A) and a 2- or 5-kDa tetra-amino PEG (Prepolymer B; Supporting Information Table S1). The cleavable crosslinkers and tissue adherent HSC groups of the PRO-L series contained *N*-methyl-*N*-methoxyethyl sulfonamide (MMESA) as the pK_a modulator which has a cleavage $t_{1/2}$ ~50 days *in vitro* at pH 7.4, 37°C. From previous studies[4] we expect the *in vivo* cleavage $t_{1/2}$ to be twofold to threefold lower, or ~18 to 25 days. For synthesis of Prepolymer A in the PRO series (Scheme 4), PEG_{20kDa}-$[NH_2]_8$ was treated with succinic anhydride, and the resultant PEG_{20kDa}-$[NHCO\text{-}CH_2CH_2\text{-}CO_2H]_8$ was activated with

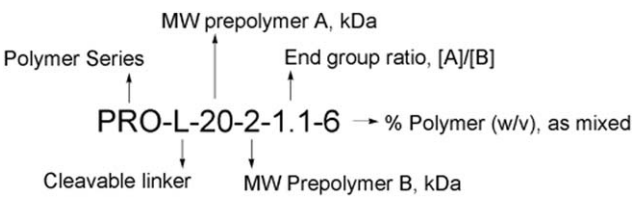
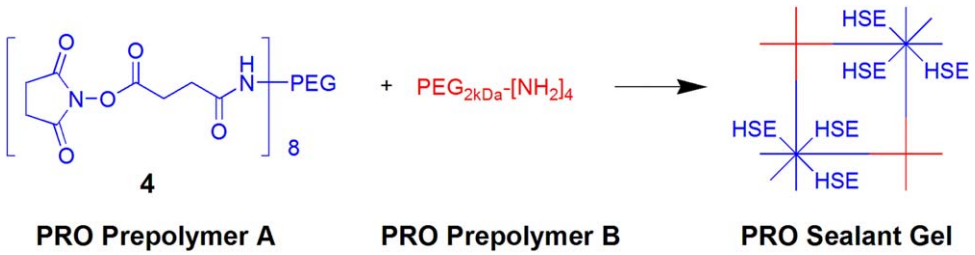
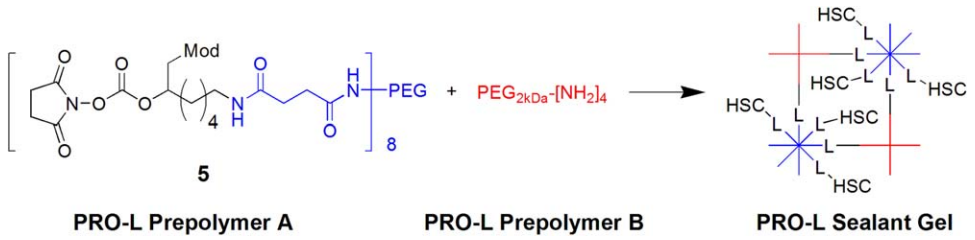


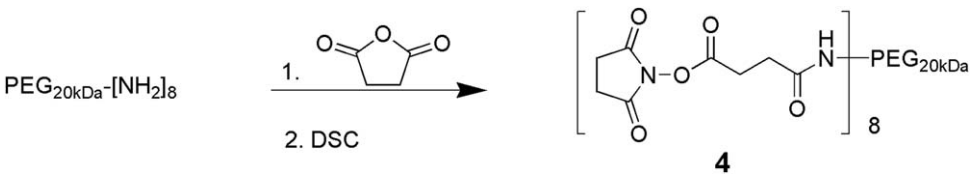
FIGURE 1. Nomenclature describing sealant gel formulas.



SCHEME 2. Formation of a PRO sealant gel.



SCHEME 3. Formation of a PRO-L sealant gel.



SCHEME 4. Synthesis of the PRO series Prepolymer A.

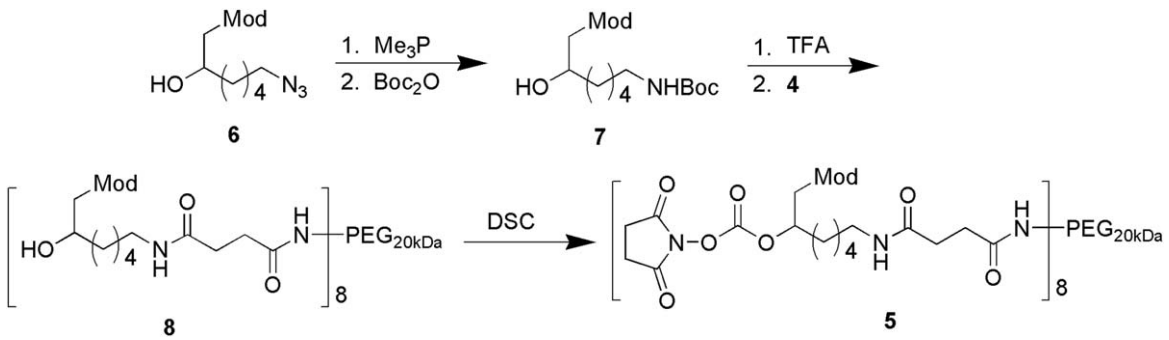
disuccinimidyl carbonate (DSC) to give the PEG_{20kDa}-[NHCO-CH_2CH_2-CO-HSE]$_8$, **4**. The same procedure was used to prepare the analogous prepolymer containing 40-kDa PEG.

For synthesis of Prepolymer A in the PRO-L series (Scheme 5), azide **6** was reduced to the corresponding amine, which was treated with di-tBu-dicarbonate to give **7**. After purification, **7** was treated with trifluoroacetic acid (TFA), and the resultant amine was acylated with PEG_{20kDa}-[HSE]$_8$, **4,** to give **8**. Finally, treatment of **8** with DSC gave the activated Prepolymer A **5.**

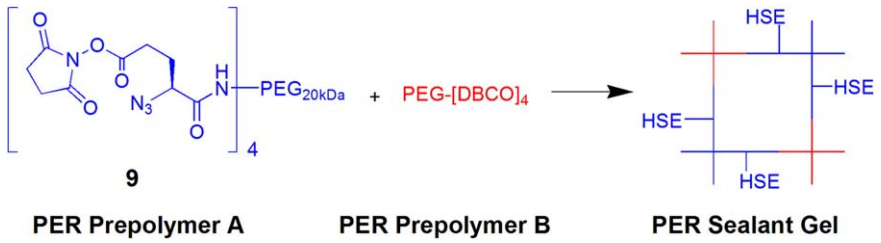
PER and PER-L series

The PER sealant gels (Scheme 6) were prepared by polymerizing a 20-kDa 4-arm PEG with bi-functional end groups having a total of 4 HSE and 4 azides (Prepolymer A) with 5- and 20- kDa PEG-[DBCO]$_4$ (Prepolymer B). The PER-L sealants (Scheme 7) used 5-, 10-, or 20-kDa 4-arm PEG with 4 azide and 4 HSE end groups (Prepolymer A) and 5-, 10-, or 20-kDa PEG-[DBCO]$_4$ (Prepolymer B), as specified in Supporting Information Table S1. After the azido-cyclooctyne crosslink coupling, 4-HSE end groups remain for tissue adhesion. As in the PRO-L gels, the PER-L series also contain cleavable crosslinks that use MMESA as the pK_a modulator.

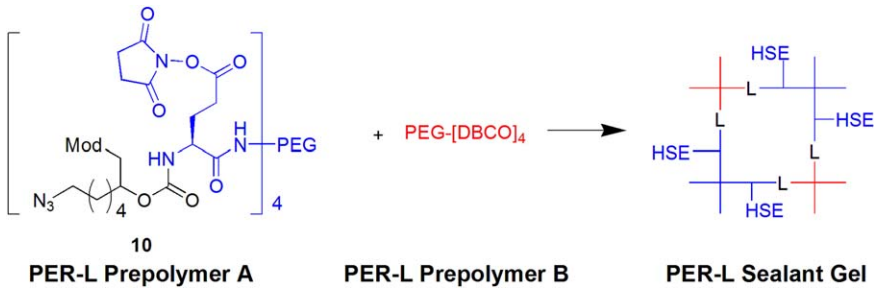
For the synthesis of Prepolymer A in the PER series (Scheme 8), PEG-[NH_2]$_4$ was first acylated with N_3-Glu(OtBu)-OSu to give [N_3-Glu(OtBu)-NH]$_4$-PEG_{20kDa}, **11**. After treatment of **11** with TFA the corresponding acid was converted to an HSE using DSC to give Prepolymer A [N_3-Glu(OSu)-NH]$_4$-PEG_{20kDa}, **9**.



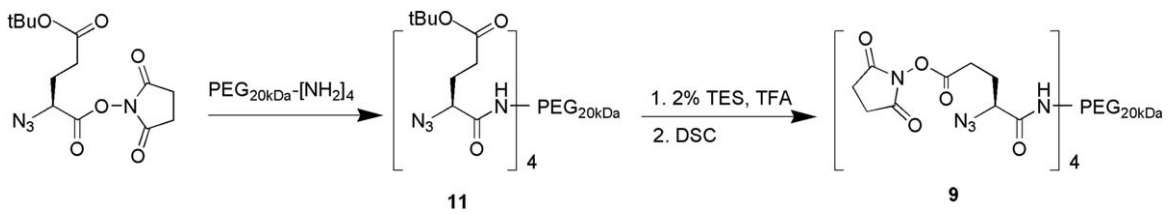
SCHEME 5. Synthesis of a PRO-L series Prepolymer A.



SCHEME 6. Formation of a PER sealant gel.



SCHEME 7. Formation of a PER-L sealant gel.



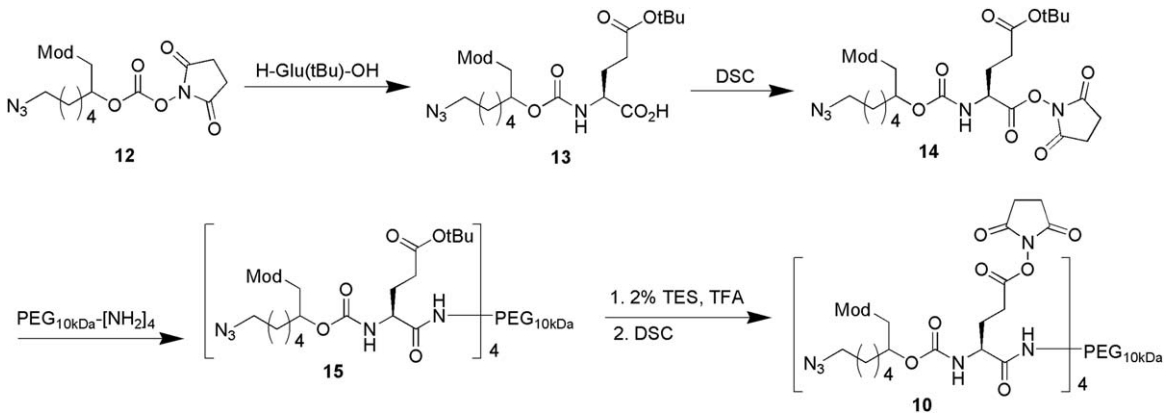
SCHEME 8. Synthesis of a PER series Prepolymer A.

Scheme 9 shows the synthesis of the 10-kDa PER-L Prepolymer A. The azido-linker HSC **12** was used to acylate H-Glu(tBu)-OH to give the azido-Linker-Glu(tBu)-OH **13**, which was activated to its HSE **14** and attached to PEG_{10kDa}-$[NH_2]_4$ to give **15**. Removal of the tBu ester of **15** with TFA and conversion of the intermediate carboxylic acid to the HSE gave the PER-L Prepolymer A $[N_3$-L(MMESA)-Glu(HSE)-NH$]_4$-PEG_{10kDa} (**10**). The corresponding 5- and 20- kDa Prepolymers A were prepared in the same manner.

RESULTS

Dependence of gelation time on pH for the PRO series sealants

Formation of PRO gels involves amide formation between HSEs or HSCs of Prepolymer A and free amino groups of Prepolymer B. As such, the gelation time can be varied by the concentration of components, or simply by pH which dictates the concentration of reactive free amines of Prepolymer B. Sealant gel PRO-L-20–2-1.1–6 gel was chosen for



SCHEME 9. Synthesis of a PER-L series prepolymer A.

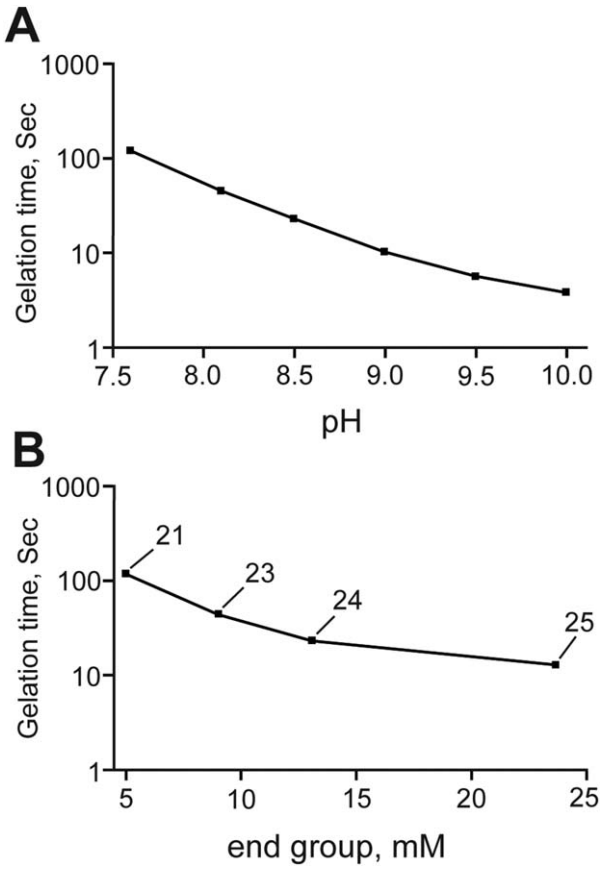


FIGURE 2. (A) Gelation time of a PRO series gel (PRO-L-20–2-1.1–6) as a function of pH. (B) Gelation time of four PER-L series gels as a function of the Prepolymer A end group concentration; annotations refer to entry numbers in Supporting Information Table S1.

study because it was most similar to Duraseal Xact in swelling and strength (see below). Here, equal volumes of a freshly prepared solution of 10.4% 24-kDa Prepolymer A (4.4 m*M*) in water (or 10 m*M* P_i, pH 4, and sufficient NaCl for isotonicity), and 1.5% of 2.0-kDa Prepolymer B (7.5 m*M*) in 100 m*M* of an appropriate buffer at pH 7.6 to 10 were rapidly mixed, and gelation time visually measured (Supporting Information IV). Figure 2(A) shows the time to gelation of a PRO-L sealant (PRO-L-20–2-1.1–6) over the range of pH 7.6 to 10. As shown, gelation time varied from $\sim$ 120 s at pH 7.6 to $\sim$4 s at pH 10.0. As a control, Duraseal showed gelation times of 200 s at pH 7.5 and $\sim$2.5 s at pH 9.5. In principle, the rate could have been increased further without additional swelling by using smaller PEGs in the prepolymers but faster gelation times were not necessary.

Concentration dependence of gelation time for the PER series sealants

The rate of the pH independent SPAAC reaction used for gel polymerization in the PER series can be modified by varying the concentration of prepolymer end groups. To avoid increased swelling associated with PEG content and yet vary the end group concentration, we varied the size of the prepolymers while keeping the ratio of end groups at 1.0. Thus, we mixed equal molar amounts of each of the 20-kDa prepolymers to obtain 5 m*M* of each end group, each of the 10-kDa prepolymers to obtain 9 m*M*, each of the 5-kDa prepolymers to obtain 24 m*M*, and the 10-kDa prepolymer A, and 5-kDa prepolymer B to obtain 13 m*M*. As shown in Figure 2(B), the gelation times of these PER-L series gels span 120 s at 5 m*M* end groups to 13 s at 24 m*M* end groups.

Gel swelling as a function of ideal crosslink density.

The dominant factor affecting the concentration of gels at equilibrium swelling is the crosslink density, ρ_x, defined as the concentration of elastically effective chains; in the present case, $\rho_x = 4$ * the concentration of the limiting reactant, Prepolymer B. Calculated as such ρ_x would be the ideal crosslink density assuming all arms of the prepolymer B have reacted with prepolymer A. A plot of ρ_x vs. the mass swelling ratio, Q (the ratio of swollen gel mass to dry mass, Supporting Information Table S1), revealed a nonlinear concave-downward plot (Supporting Information Figure S2) as previously reported for similar PEG hydrogels.[9] In this work, we related ρ_x to the ideal mole fraction of crosslinks (MFC) per ethylene oxide unit (-$(CH_2)_2$O-) of PEG; that is, MFC = ρ_x/[-$(CH_2)_2$O-]. Here, [-$(CH_2)_2$O-] was calculated as:

$$[-(CH_2)_2O-] = ([\text{Prepolymer A}] * MW_{PEG\_A} + [\text{Prepolymer B}] * MW_{PEG\_B})/44 \quad (1)$$

The MFC was altered by changing the [-$(CH_2)_2$O-] using PEG prepolymers of varied MW between 2 and 20 kDa in the PER and PRO series and, for the PRO series, by also adjusting the stoichiometry of cross-linking end groups (i.e., [HSE]/[amines] = 1 to 2).

For surgical sealants, the swelling ratio of the formulation is the difference in volume of the initially prepared gel (V_i) and the volume after equilibrium swelling (V_S). This parameter is especially important for gels that require minimal swelling to avoid expansion in a confined space (e.g., dural sealants). The formulation swelling ratio (V_S/V_i) was determined as postswelling weight/unswollen weight of the gel, equivalent to the amount of water uptake during swelling of the initially formed gel. The % polymer at equilibrium swelling was calculated as the (initial % polymer)/(V_S/V_i). The swelling data of all gels studied here are provided in Supporting Information Table S1. A plot of MFC vs. % polymer at equilibrium swelling [Figure 3(A)], shows a linear correlation ($R^2 = 0.637$) for both PER and PRO series gels with or without cleavable linkers. Thus, as described for related PEG gels, the MFC is directly related to the overall water content, and can be used to predict the equilibrium swelling.

Figure 3(B) shows the formulation swelling ratios (V_s/V_i) of the marketed sealants Coseal, Duraseal and Duraseal Xact as controls, as well as six selected PRO and PER gels from Supporting Information Table S1 that exhibit low formulation swelling ratios and high burst pressures [see below, Figure 4(B)] comparable to or better than the controls. All six gels swelled less than Coseal, five less than Duraseal, and one showed no swelling as observed with Duraseal Xact. The

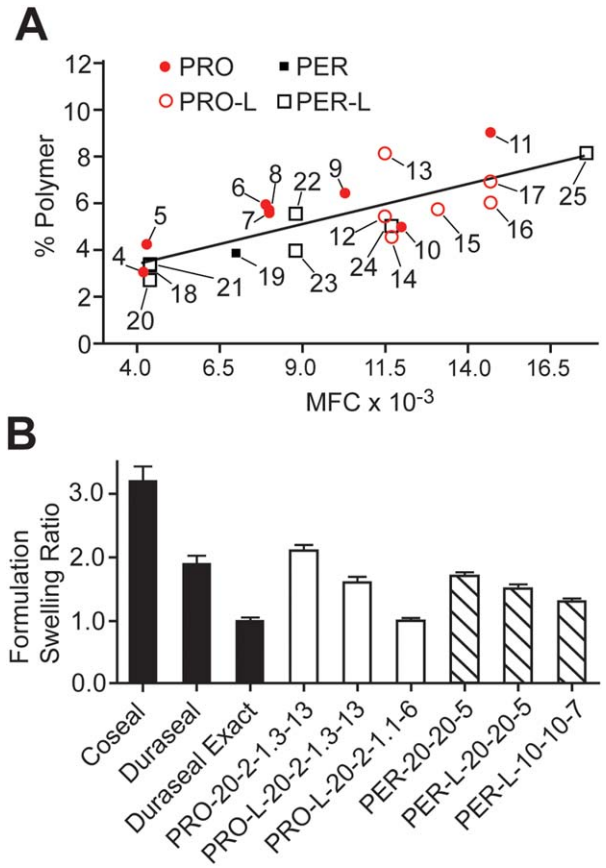


FIGURE 3. Effect of mole fraction of crosslinks (MFC) on gel swelling. (A) Plot of % Polymer at equilibrium swelling vs. MFC of all gels studied, from Supporting Information Table S1, $R^2 = 0.637$. (B) Formulation Swelling ratio of control marketed sealants (solid) three PRO gels (open) and three PER gels (hatched) selected from this study.

presence of a cleavable linker as in PRO-L-20–2-1.3–13 and PER-L-20–20-1.0–5 shows slightly diminished swelling compared to gels with stable crosslinks.

Burst pressure as a function of ideal crosslink density

For burst pressure measurements, 32 mm diameter collagen sausage casing disks with a 3 mm hole in the center were soaked in PBS and placed in a 13 mm gel-disk mold; sealant disks (2 × 13 mm) were formed over the hole-defect using 270 μL of a freshly prepared solution of gel prepolymers. To measure a minimal time to reach the maximum strength, we prepared a PRO-L-20-1.1–6 gel with Prepolymer B at pH 7.6 [gelation time ~120 s; Figure 2(A)], and assessed burst pressure as a function of time. The gel reached maximum strength by 15 min, which was well before the first time of measurement (30 min) used for other gels. Sealants were cured for 30 min then were transferred to the burst-pressure apparatus (ASTM F 2392–04) to measure burst pressure. Samples were also allowed to swell for 18 hr in PBS before burst pressure assessment. Cohesive failure is defined as a burst through the gel center, and adhesive failure refers to gel detachment from the collagen membrane. A control PER gel lacking covalent bond-forming adhesive groups showed immediate adhesive failure on applying minimal pressure. With this exception, adhesive failures were not observed in either unswollen or swollen PER and PRO gels prepared here, indicating the adhesion reaction is rapid and effective; further, in the PRO series, a 10% excess HSE over amine showed equally good adhesion as a 50% excess. Supporting Information Table S1 shows burst-strength values for all gels studied here. Figure 4(A) shows a linear correlation between the burst pressure of swollen gels and % polymer at equilibrium swelling (slope = 0.54, $R^2 = 0.765$). Burst pressures of the pre-swollen gels (Supporting Information Table S1) were slightly lower or equal to those observed after swelling, but showed a similar correlation (slope = 0.47, $R^2 = 0.718$; not shown). Hence, burst pressure can be predicted from swollen polymer concentration.

Figure 4(B) shows the burst-pressures of Coseal, Duraseal, and Duraseal Xact, as well as the six selected PRO and PER gels from Figure 3(A). With exception of Duraseal Xact, none

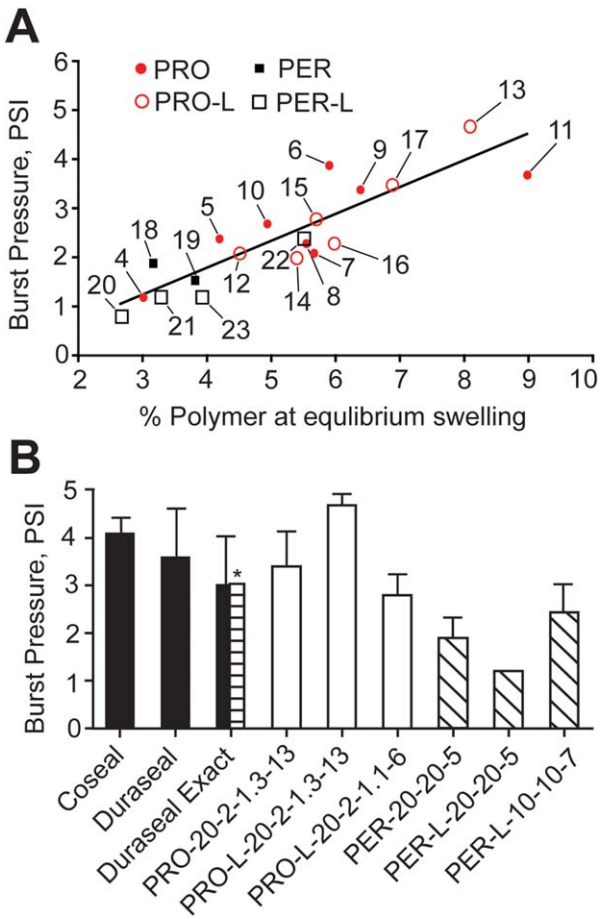


FIGURE 4. Burst pressures of sealant gels. (A) Plot of post-swelling cohesive burst pressure vs. % polymer at equilibrium swelling of all gels studied, from Supporting Information Table S1, $R^2 = 0.765$. (B) Burst pressure of control marketed sealants (solid), three PRO gels (open) and three PER gels (hatched) selected from this study. All were cohesive failures except for the indicated adhesive failure with Duraseal Exact (striped,*).

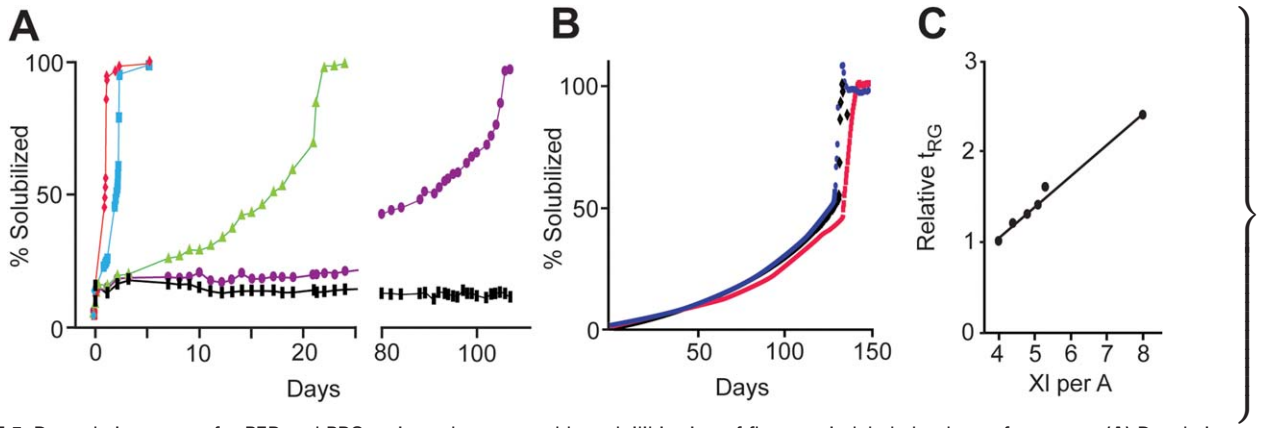


FIGURE 5. Degradation curves for PER and PRO series gels measured by soluilibization of fluorescein labeled polymer fragments. **(A)** Degelation of gels analogous to the PER series sealants as a function of the p*K*a modulating group used in the cleavable crosslinker: $ClPhSO_2-$ (♦), $PhSO_2-$ (■), $O(CH_2CH_2)_2NSO_2-$ (▲),−CN (●), no modulator (●).[3] **(B)** Degelation of PRO series sealant PRO-L-20–2-1.1–6, the three curves (red, black and blue) are triplicate measurements. Degelation measurements at pH 9.4 were converted to days at pH 7.4 based on the relationship $t_{7.4} = t_{9.4}*10^{(9.4\text{-}7.4)}$.[3] **(C)** dependence of t_{RG} on the number of arms cross linked on the 8 armed prepolymer A (Xl per A) of a PRO style gel.[3]

of the gels shown in Figure 4(A) or listed in Supporting Information Table S1 showed adhesive failures. The frequency of Duraseal Xact adhesive failures was 75% for preswollen gels and 25% for swollen gels. We speculate that Duraseal Xact may not have or, as a dural sealant, need a high level of reactive adhesive groups available, but the exact composition is not available to us. Although Coseal shows one of the highest burst pressures it is also shows highest swelling [Figure 3(B)]. We note that the burst pressure for Coseal is higher than reported using collagen membrane substrates.[10,11] All of the gels described here behave favorably with the comparators. The higher burst pressures observed with the PRO vs. the PER series are likely due to the higher amount crosslinking possible with the eight reactive end-groups of the Prepolymer A component.

Gel degradation

The gel syntheses described here were designed to permit installation of β-eliminative carbamates in the crosslinks. We have previously shown that PEG hydrogels containing such linkers undergo predictable specific base-catalyzed degradation based on the pK_a modulator used;[3] Figure 5(A) shows reported examples of degelation curves of gels analogous to the PER series of sealants, determined by measuring solubilization of fluorescein labeled gel fragments.[3] As shown, the time to reverse gelation (t_{RG}), as defined as the time when 100% of the gel becomes soluble, varies from ~ 1 to 100 days depending on the pK_a modulator used in the cleavable linker.[3] Figure 5(B) shows the degradation profile, determined using the same assay (Supporting Information **IV**), of a high-strength low-swelling PRO gel (PRO-L-20–2-1.1–6) that contains β-eliminative cleavable crosslinks. In the PRO series t_{RG} is related to the $t_{1/2}$ of the linker as well as the number of crosslinks per 8-arm Prepolymer A [Figure 5(C)].[3] The MMES modulator used confers an *in vitro* $t_{1/2}$ of ~1500 h for the linker and, since 7.2 of the 8-arms of Prepolymer A are used in crosslinks [Figure 5(C)], this gel has a t_{RG} of 129 ± 5 days.

Stiffness and thermodynamic properties of model tetra-PEG-hydrogels

Figure 6(A) shows a plot of the shear modulus (G) obtained by elongation testing (Supporting Information **IV**) vs. prepolymer concentration for three hydrogels: (a) a previously reported tetra-PEG hydrogel made from a 4-arm tetra-amino PEG_{20kDa} and a tetra-HSE PEG_{20kDa}, analogous in network configuration to the PER series.[6,7,13] (b) a Tetra-PEG hydrogel made from a 4-arm PEG_{20kDa} with DBCO end groups and tetra-azido PEG_{20kDa}, directly analogous to the triazole cross-linked PER series; and (c) a hydrogel prepared from 8-arm PEG_{40kDa} with DBCO end groups previously reacted with four equivalents of PEG_7-azide and a tetra-azido PEG_{20kDa}, analogous to the PRO series sealants. The data in Figure 6(A) can be used to calculate the Young's modulus (E) as $E = G^*$ 3. Using the phantom network model, $G = \xi_x RT$ where ξ_x is the molar concentration of cycle rank, R is the universal gas constant, and T is the absolute temperature, we calculated ξ_x from G that was obtained by elongation testing, and plotted these values vs. prepolymer concentration [Figure 6(A)].

Assuming that the polymer network of hydrogels behaves as those in the phantom network model, we employed a modified version of the Flory–Rehner equation [Eq. (2)][12]

$$\xi_x = -\frac{\ln(1-\phi)+\phi+\chi\phi^2}{V_1\left(\frac{\phi}{\phi_0}\right)^{1/3}} \quad (2)$$

where ϕ is the polymer volume fraction in the equilibrium state, ϕ_o is the polymer volume fraction in the preparation stage, χ is the Flory–Huggins parameter, and V_1 is the molar volume of the solvent. Using this equation, we determined Flory–Huggins parameter (χ) of Tetra-PEG hydrogels that were cross-linked by triazole bonds to be 0.484 ± 0.0015 for the PER style gel and 0.483 ± 0.0013 for the PRO style gel [Figure 6(B)]. It can be seen that the triazole crosslinked gels have similar elasticity and swelling properties, regardless of the number of arms, suggesting that the nonreactive arms do

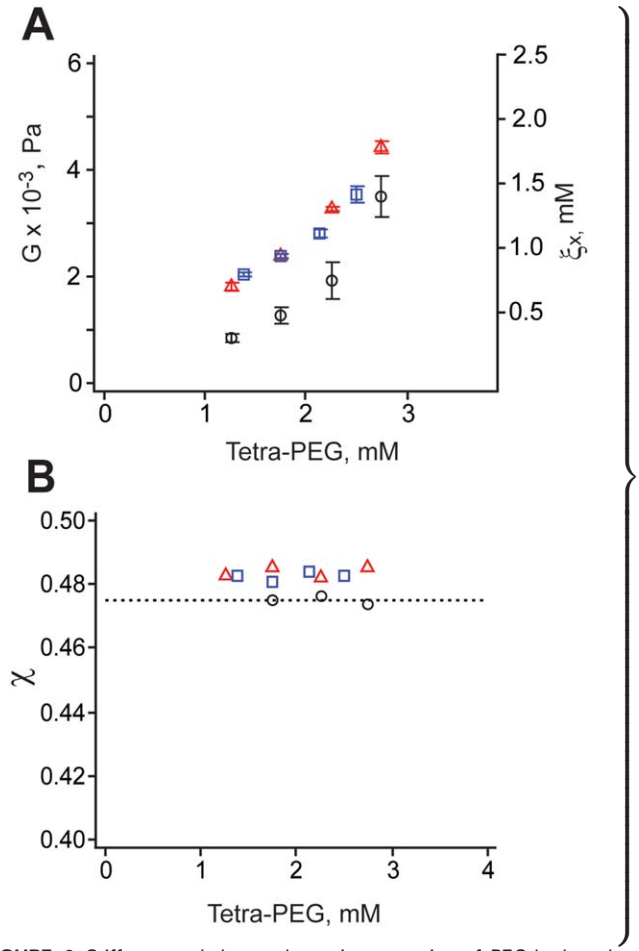


FIGURE 6. Stiffness and thermodynamic properties of PEG-hydrogel models of surgical sealants. **(A)** Shear modulus (G) and cycle rank (ξ_x) of Tetra-PEG hydrogels cross-linked by amide bonds (○) or triazoles (△), or triazole crosslinked gels prepared from the partially capped 8-arm PEG and 4-arm PEGs (□) as a function of prepolymer concentration. **(B)** Flory–Huggins parameter (χ) of the polymer network of Tetra-PEG hydrogels described in Panel A. The dotted line represents the reported value for Tetra-PEG hydrogels crosslinked with amide bonds ($\chi = 0.475 \pm 0.0010$).[12]

not influence the physical properties. Also, these parameters are slightly higher than those determined for the well-studied Tetra-PEG hydrogels;[7,8] the higher Young's modulus is due to the high yield of the SPAAC crosslinking reaction, and the higher Flory–Huggins parameter might be due to the hydrophobic dibenzotriazole moieties in the crosslinks.

DISCUSSION

In this work, we prepared and characterized two different formats of PEG-based hydrogels—termed the PRO and PER series—as potential surgical sealants. Within each series similar chemistries are used, and strength and swelling properties are conferred by different sizes and concentrations of the prepolymers.

The PRO series of sealants is most similar to the approved PEG-based hydrogel sealants—Coseal, Duraseal, and Progel. For these marketed sealants, a multiarm PEG containing reactive HSE or HSC end groups is rapidly mixed with a second polymer containing multiple thiol or amine nucleophiles—tetra-thiol PEG in Coseal, tri-lysine in Duraseal and albumin in Progel. The PRO series, sealants were made by polymerization of two PEG prepolymers, one with eight-reactive HSE end-groups and another with four amine end-groups. Polymerization of the 4-arm amino PEG with an excess of the HSE-containing polymer results in a gel with a network of amide crosslinks; the remaining unreacted HSE groups in the gels are used for tissue adherence. In the PER series, one prepolymer has four HSC and four azide end groups, whereas the other has four cyclooctyne end groups that specifically connect to the four azides by SPAAC. On mixing the prepolymers, equimolar end-groups react stoichiometrically to form a Tetra-PEG hydrogel crosslinked by dibenzotriazole connections.

The original well-characterized Tetra-PEG gels have amide crosslinks and are characterized by a homogeneous network structure, high deformability and mechanical strength.[7,8] We have shown that the Young's modulus and Flory–Huggins parameter of the PER series Tetra-PEG gels are equivalent to the amide-cross-linked Tetra-PEG gels, so it appears that the hydrophobic connecting groups have little effect on gel quality. Interestingly, gels similar to the PRO series formed by connection of an 8-arm PEG with a 4-arm PEG by dibenzotriazole linkages also have nearly identical elasticity and Flory–Huggins parameters to the Tetra-PEG gels. The elasticity of a surgical sealant is an important property when the intended use is to seal vessels or organs with high mobility—lungs, skin, blood vessels and heart tissue—and the elastic modulus should be similar to that of the surrounding tissue. The Tetra-PEG gels reported here are significantly softer than the comparator sealants: Coseal and Duraseal have reported Young's modulus (E) of 70 to 100 kPa, whereas our PER gel has an E of 6 to 13.5 kPa.[10,14]

The gelation time of a surgical sealant must be sufficiently short to avoid excess run-off from the site of application. For a crosslinking reaction with a given rate constant, gelation time is controlled by the concentration of reactant end-groups in the mixed solution of prepolymers. Since the PRO series gels involve amide formation between HSEs or HSCs of Prepolymer A and free amino groups of Prepolymer B, the gelation time can be controlled by the pH of the mixed solutions. At pH 10, Prepolymer B has a high concentration of free vs. protonated amines, and rapidly reacts with the HSE groups of Prepolymer A; for example, PRO-L-20–2-1.1–6 has a rapid gelation time of $\sim$ 4 s, slightly longer than that of comparators Duraseal, Coseal, and Proseal.[15] In contrast, the polymerization rate in the PER series is pH independent, and the gelation time is modified by varying the concentration of prepolymer cyclooctyne and azide end groups. The fastest polymerizing PER gel was obtained by mixing equimolar amounts of 5-kDa prepolymers to obtain 24 mM end groups, which gave a gelation time of 13 s. The gelation time for such gels can be further reduced by partial pre-crosslinking of components before mixing the amounts required for gel formation (e.g., premixing a 4:1 end group ratio of PER components will reduce

gelation time approximately twofold). While gelation times of the PER series were not as fast as the PRO series, they are likely sufficient for most purposes. In principle, the gelation rate of either the PRO or PER series could be further increased by reducing the size of the prepolymer components to allow increases of the end-group concentrations.

Burst pressure test results of six optimized PER and PRO sealants on collagen membranes suggest that the sealants in Figure 4 should be effective in sealing blood vessel leaks. Blood pressure in humans ranges from about 0.48 PSI (25 mm) for capillary bleeding to 3.9 PSI (202 mm) for extreme hypertension. The gels sustained pressures of ~0.6 to 2.5 PSI (~31 to 129 mm) in the PER series, and ~3.0 to 5.0 PSI (~155 to 259 mm) in the PRO series for a 3-mm diameter orifice defect on collagen membranes, much larger than the $\leq$1 mm diameter of commonly used sutures. The burst pressures of our gels compared favorably with the comparator gels Duraseal, Duraseal Xact, and Coseal, even though we obtained a higher value than reported for the latter;[10] Progel, a pleural sealant, had a burst pressure of ~80 mm in a rat-lung incision model.[15] Hence, the gels described here have similar or higher burst pressures than the PEG-based comparators. The burst pressure of the PRO and PER sealants showed a linear correlation with the mole fraction of crosslinks. Of six sealants tested, the PRO series showed higher burst pressure than the PER series. Because Prepolymer A of the PRO series has 8 arms, the gels can have a greater mole fraction of crosslinks which strengthens the gels.

The burst pressure test on collagen membranes also provided an indication of the adhesive strength of the gels. PER and PRO gels failed solely by rupturing, that is, cohesively; adhesive failures were not observed in either preswollen or swollen gels, indicating that the amide-forming adhesion reaction of our gels is both rapid and effective.

Different sealant applications may have different tolerances for postapplication swelling. As examples, sealants used in the abdomen can tolerate significant swelling, whereas dural sealants require minimal swelling to avoid neural compression. Studies of several PEG polymer systems have shown a covariance of the crosslink density and shear moduli and an inverse correlation with swelling.[1,9] Here, we show covariance of the mole fraction of crosslinks, gel concentration and burst pressure in both the PRO and PER gel series, as well as a correlation of Young's modulus with gel concentration. We varied the mole fraction of crosslinks of the PRO and PER gels by using PEG prepolymers of different sizes and/or by adjusting the stoichiometry of crosslinking end groups. The inverse linear correlation of gel content at equilibrium swelling and the mole fraction of crosslinks for both PER and PRO series gels showed that the MFC is a useful predictor of equilibrium swelling. Our low-swelling Gels that also had high burst pressures swelled less than Coseal or Duraseal, and one showed essentially no swelling, as observed with Duraseal Xact.

Degradation rate is an important property of a surgical sealant.[1] The sealant must remain intact for a sufficient period to allow healing, but not so long that it interferes with later phases of the healing process. The comparator PEG-based hydrogels—Coseal, Duraseal, and Progel—all contain ester groups in the crosslinks which hydrolyze to cause gel degradation and concomitant decrease in strength. However, the degradation rate of ester-crosslinked gels is unpredictable and is empirically established by trial and error variation of the number of crosslinks. A novel aspect of the currently described gels is the ability to incorporate self-cleaving β-eliminative linkers into the crosslinks of the polymer that control the rate of biodegradation. Assuming there are no long-duration pH perturbations—which can modify crosslink cleavage rates—the time of gel degradation may be predictably controlled to occur over days to months as needed for a particular application. To our knowledge, this feature has never before been achieved in a sealant. Since numerous β-eliminative linkers with different degradation rates are available,[3,4] it should be a straightforward task to design a surgical sealant/anti-adhesive with any desired rate of dissipation.

β-Eliminative linkers have also been used for releasable attachment of drugs to Tetra-PEG hydrogels.[3] For example, a cleavable amino-linker-drug with a shorter half-life than that used in crosslinks can be coupled with excess HSC groups of Prepolymer A of the PRO or PER series. After application, the drug would be slowly released, followed by gel degradation.

In summary, we prepared and characterized two types of PEG-based hydrogels as potential surgical sealants and/or anti-adhesives. Our studies revealed correlations of parameters relevant to sealants—for example, gelation time, swelling, gel strength—vs. easily controllable variables—for example, concentration of components and mole fraction of crosslinks. As a consequence, simple variations of the formats can be designed *a priori* to meet criteria for several specific applications. Variations of the two gel formats provided properties similar to any of the PEG-based surgical sealants/anti-adhesives approved by the FDA and currently marketed. In addition, the gels meet criteria for ideal ophthalmic adhesives[1,16] and are related to Resure Sealant, the first FDA-approved material for sealing corneal incisions after cataract surgery. A unique feature of the present sealants is the ability to incorporate self-cleaving β-eliminative linkers into crosslinks of the gel that allow controlled degradation that is tunable to the healing requirements of affected tissue. Similar linkers could also easily be used to tether drugs to the sealant that could be released over a predictable period.

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