

Hydrogel drug delivery system with predictable and tunable drug release and degradation rates

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Many drugs and drug candidates are suboptimal because of short duration of action. For example, peptides and proteins often have serum half-lives of only minutes to hours. One solution to this problem involves conjugation to circulating carriers, such as PEG, that retard kidney filtration and hence increase plasma half-life of the attached drug. We recently reported an approach to half-life extension that uses sets of self-cleaving linkers to attach drugs to macromolecular carriers. The linkers undergo β -eliminative cleavage to release the native drug with predictable half-lives ranging from a few hours to over 1 y; however, half-life extension becomes limited by the renal elimination rate of the circulating carrier. An approach to overcoming this constraint is to use noncirculating, biodegradable s.c. implants as drug carriers that are stable throughout the duration of drug release. Here, we use β -eliminative linkers to both tether drugs to and cross-link PEG hydrogels, and demonstrate tunable drug release and hydrogel erosion rates over a very wide range. By using one β -eliminative linker to tether a drug to the hydrogel, and another β -eliminative linker with a longer half-life to control polymer degradation, the system can be coordinated to release the drug before the gel undergoes complete erosion. The practical utility is illustrated by a PEG hydrogel–exenatide conjugate that should allow once-a-month administration, and results indicate that the technology may serve as a generic platform for tunable ultra-long half-life extension of potent therapeutics.

click chemistry | regenerative medicine | tetra-PEG

Conjugation of drugs to macromolecular carriers is a proven strategy for improving pharmacokinetics. In one approach, the drug is covalently attached to a long-lived circulating macromolecule—such as PEG—through a linker that is slowly cleaved to release the native drug (1, 2). We have recently reported such conjugation linkers that self-cleave by a nonenzymatic β -elimination reaction in a highly predictable manner, and with half-lives of cleavage spanning from hours to over a year (3). In this approach, a macromolecular carrier is attached to a linker that is attached to a drug or prodrug via a carbamate group (1; Scheme 1); the β -carbon has an acidic carbon–hydrogen bond (C–H) and also contains an electron-withdrawing “modulator” (Mod) that controls the pK_a of that C–H. Upon hydroxide ion-catalyzed proton removal to give 2, a rapid β -elimination occurs to cleave the linker-carbamate bond and release the free drug or prodrug and a substituted alkene 3. The rate of drug release is proportional to the acidity of the proton, and that is controlled by the chemical nature of the modulator; thus, the rate of drug release is controlled by the modulator. It was shown that both in vitro and in vivo cleavages were linearly correlated with electron-withdrawing effects of the modulators and, unlike ester bonds commonly used in releasable linkers (2), the β -elimination reaction was not catalyzed by general bases or serum enzymes. Although the PEG-Michael acceptor products are potentially reactive toward nucleophiles, their reactivities correlate with their rates of formation and are in general slow compared with renal elimination (3).

The in vivo elimination rate of a drug (k_{drug}) released from a circulating carrier is described by $k_{\text{drug}} = k_1 + k_3$, where k_1 is the rate of linker cleavage and k_3 is the rate of clearance of the conjugate (3). With very slowly cleaving linkers, the limitation to

half-life extension is therefore not the cleavage rate (k_1) of the linker, but rather the elimination rate (k_3) of the circulating conjugate; for PEG conjugates, the elimination $t_{1/2}$ is usually no longer than ~ 7 d in humans (4, 5). To overcome this limitation, we used our linkers to conjugate the 39 amino acid peptide exenatide to a noncirculating, nondegradable polymeric hydrogel (3); here, k_3 is negligible so the overall elimination rate of the drug, k_{drug} , approaches the rate of linker cleavage, k_1 . Indeed, when the hydrogel was implanted s.c. in rats, the half-life of the peptide in serum was increased 150-fold over that for a bolus injection. Although this demonstrated the utility of the β -eliminative linkers for in vivo s.c. implants, the gel used was nondegradable, and a bio-compatible, biodegradable implant is essential for practical use.

Most biodegradable drug-delivery implants encapsulate a drug within gels of limiting pore size that retards diffusion, and release the drug concomitant with hydrolytic degradation—often of ester bonds—of the polymer (6, 7) (Fig. 1A). In contrast, for our drug-releasing linkers, we required a porous gel that readily allows diffusion, is substantially stable over the duration of drug release, and degrades subsequent to release of the drug (Fig. 1B). Although conventional ester-containing degradable gels could serve as a scaffold, ester linkages per se do not provide wide variation in cleavage rates, and it is difficult to achieve tunable, slow gel erosion rates without modifying gel network properties. It was our desire to develop a system in which we could keep gel properties largely constant, and achieve different degradation rates by modifying the rate of gel scaffold cleavage. In the present work, we tether drugs or drug surrogates to large-pore hydrogels prepared from two multi-arm PEG macromonomers using β -eliminative linkers that cleave at desired rates of drug release; similar β -eliminative linkers having slower cleavage rates are used to cross-link four arms of each macromonomer to form Tetra-PEG gels (8, 9). In this manner, either or both cleavage rates can be tuned to coordinate drug release and gel degradation without introducing major structural differences in the gels.

Results

Design and Synthesis of Hydrogels. PEG was chosen as a hydrogel scaffold because star polymers for step-growth polymerization are available, precise chemical modifications are facile, it is bio-compatible, and is a Generally Recognized as Safe substance. The ~ 7 -nm (10 kDa PEG) distance between nodes of the gels used here provides a porous gel (mesh size > 5 nm) that for our purposes would not significantly retard diffusion of proteins of up to at least 100 kDa (10, 11). The general approaches used for tethering drugs to hydrogels and for hydrogel syntheses are depicted in Scheme 2

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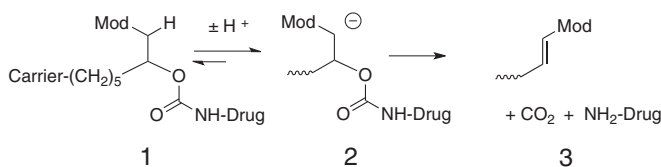
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Scheme 1.



and Fig. 2 and detailed in *SI Text, Preparation of End-Group Modified Multivalent PEGs*. 6-Azidohexylcarbamoyl-hydroxysuccinide or azido-linker succinimido carbonates (HSC) **4** possessing one of the modulators **A–D** were reacted with a four-arm amine-derivatized PEG_{20kDa} to provide stable $PEG_{20kDa}(L-N_3)_4$ and cleavable $PEG_{20kDa}(L-Mod-N_3)_4$, **5**. Likewise, **4, A–D**, is reacted with an amine group of a drug to form carbamates **6, A–D**. The azido-linker drug **6** is then reacted by click chemistry with four arms of an octa-armed cyclooctyne(CO)-derivatized PEG [dibenzocyclooctyne (DBCO) (12) or bicyclononyne (BCN) (13)] to give **7**.

A key reaction used in synthesis of the hydrogels described here is Cu-free azide-alkyne click chemistry, which has become a popular method for polymerizing hydrogels (14–18). In one format (Fig. 2A), “4 × 4” hydrogels were prepared by step-growth polymerization of 20-kDa four-armed azido-PEG **5** with a stoichiometrically balanced amount of 20-kDa four-arm CO-derivatized PEG [$PEG_{20kDa}(CO)_4$] to provide a gel with 10-kDa PEG chains between nodes. Nondegradable gels used the noncleavable 6-azidohexylcarbamoyl PEG [$PEG_{20kDa}(L-N_3)_4$], and degradable gels contained β -eliminative carbamates to connect the azide and amino-PEG moieties [$PEG_{20kDa}(L2-Mod-N_3)_4$]. To monitor gel dissolution, a trace amount of absorbing and/or fluorescent “erosion” probe was covalently attached by a stable linker to CO residues before hydrogel formation (19). Although we have not studied mechanical or structural properties of these gels, analogous Tetra-PEG gels have been intensively studied and shown to possess near-ideal homogeneous polymer network properties with minimal defects (8, 9, 20). In another format (Fig. 2B), a drug or drug surrogate was tethered to the eight-arm component of a 4 × 8 hydrogel by a releasable linker. Azides of the β -eliminative carbamoyl drug **6**, a trace amount of erosion probe, and, when specified, an inert PEG cap were attached to an eight-arm CO-derivatized PEG [$PEG_{40kDa}(CO)_8$] to modify an average of four of the eight CO end-groups. Recognizing that, unlike the above 4 × 4 Tetra-PEG gels, the nonuniform modification of the number of CO end-groups and increased intramolecular coupling at low

monomer concentrations may lead to gel imperfections (19–21), the remaining end-groups were polymerized with stoichiometric amounts of $PEG_{20kDa}(L2-Mod-N_3)_4$ to give 4% wt/vol gels.

Diffusion of Molecules from Hydrogels. We determined the rates of diffusion of noncovalently bound, encapsulated proteins (MW 17.7–66 kDa) from a nondegradable 4 × 4 PEG hydrogel having 10-kDa PEG chains between nodes (Fig. 2A). Gels were prepared by reaction of $PEG_{20kDa}(L-N_3)_4$ with $PEG_{20kDa}(CO)_4$ in the presence of various proteins. Upon incubation in pH 7.4 buffer, 37 °C, the proteins diffused from the gel in the expected reverse-order of their molecular weights (*SI Text, Protein Diffusion in Encapsulating Gels*), with the largest protein (BSA, 66 kDa) diffusing much faster ($t_{1/2} \sim 2.5$ h) than the anticipated cleavage of our β -eliminative linkers; thus, diffusion is not an obstacle to controlled release.

β -Eliminative Degradation of Hydrogels. Next, we showed that hydrogels containing β -eliminative linkers within the gel network degraded in a predictable manner. PEG 4 × 4 gels were prepared that contained a trace of fluorescein erosion probe and various modulators controlling the cleavable cross-links [$PEG_{20kDa}(L2-Mod-N_3)_4$]. Gels released PEG-fluorescein fragments slowly until the reverse gelation time (t_{RGEL}), at which time the remainder of the gel rapidly and completely solubilized (Fig. 3A). As the erosion of a gel with $Mod = PhSO_2^-$ progressed, samples of the soluble fraction were analyzed by size-exclusion chromatography (SEC). In accord with degradation studies of step-growth polymers (19), released gel fragments were primarily 20-kDa monomer components at early stages, but the proportion of dimers and larger fragments increased dramatically as the reverse-gelation point neared (*SI Text, SEC-HPLC Analysis of Degrading Gel*). In an idealized step-growth network, t_{RGEL} should occur when an average of two cross-links remain attached to each node (19, 21). Thus, for the 4 × 4 gel erosions in Fig. 3A, t_{RGEL} should occur when two of the four cross-links per node are cleaved, and correlate with the $t_{1/2}$ of linker cleavage. As shown in Table 1, t_{RGEL} varied over a range of ~ 1 to ~ 100 d depending on the modulator used, and were slightly lower than, but highly correlated ($R^2 = 0.990$) with, the half-lives of β -eliminative cleavage of the same linkers in soluble PEG conjugates (3). As with cleavage rates of soluble PEG conjugates, t_{RGEL} of a 4 × 4 gel containing the $CIPhSO_2^-$ modulator was first-order with respect to hydroxide ion up to at least pH 9.0 (Fig. S1) with $k_{OH} = 5.1 \times 10^4 M^{-1} \cdot h^{-1}$. These results show that hydrogels containing β -eliminative linkers in the cross-linking chains have predictable degradation rates that can be controlled over very long periods simply by varying the modulator used.

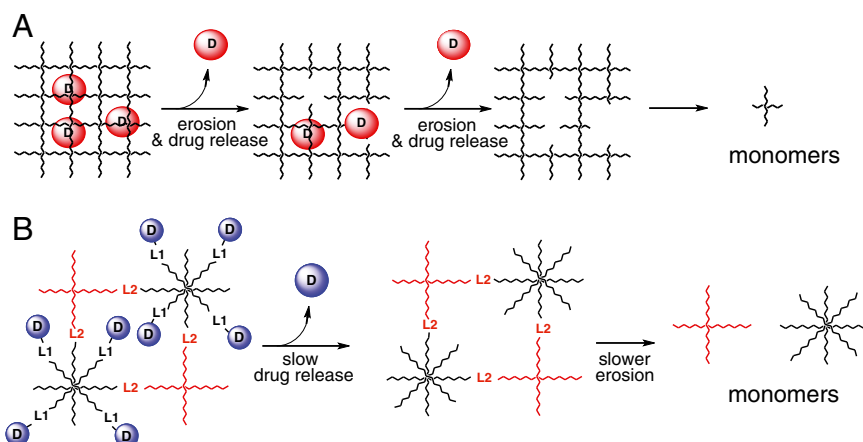
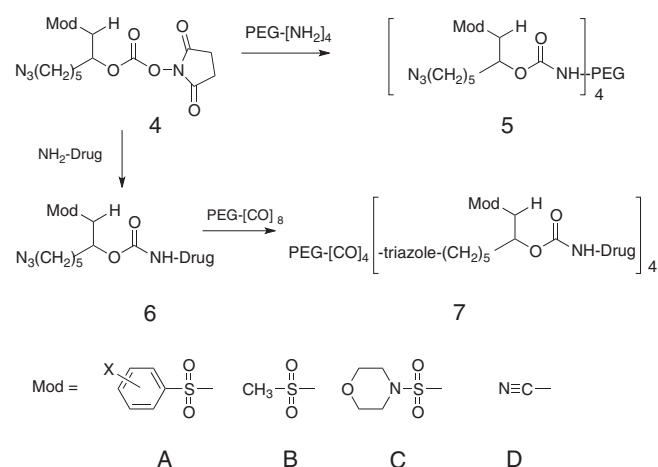


Fig. 1. Drug release from polymeric gels. (A) Encapsulated drug released concomitant with gel degradation. (B) Release by linker cleavage of covalently tethered drug, followed by gel degradation.

Scheme 2.



Tunable Drug Release with Hydrogel Degradation. To show that the technology provides predictable, tunable drug release, we studied 4×8 hydrogels that contained a surrogate drug tethered to hydrogels by β -eliminative linkers with varying cleavage rates, and a β -eliminative linker with a slower cleavage rate (Mod = MeSO₂-) to cross-link the gel. An average of four of eight end-groups of PEG_{40kDa}(DBCO)₈ were tethered to fluorescein as a drug surrogate via β -eliminative linkers, a BODIPY erosion probe, and a small PEG cap; the approximately four remaining DBCO end groups were reacted with tetra-arm PEG_{20kDa}(L2-MeSO₂-N₃)₄ to give 4×8 gels depicted in Fig. 2B. Fig. 3B shows the fluorescein drug surrogate released into solution over time. Here, the solubilized fluorescein is the sum of that released from its tether by direct cleavage at L1 of the hydrogel, and that released as PEG-fluorescein fragments upon cleavages at L2 cross-links; the latter ultimately releases free fluorescein molecules in

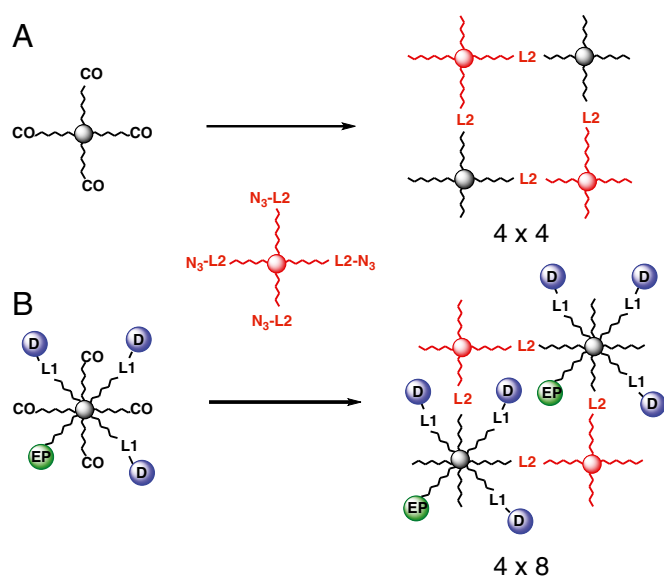


Fig. 2. Idealized hydrogels formed from four-arm PEG azide and (A) four-arm PEG-CO (4×4 gel) or (B) eight-arm PEG-CO (4×8 gel) with four available cyclooctynes. Circles represent a drug or drug surrogate (D) tethered to the network by a releasable linker, or an erosion probe (EP) connected by a stable linker; L1 is a β -eliminative linker that releases the drug, and L2 is a β -eliminative cleavable cross-link of the gel.

solution that have the same absorbance as the PEG-fluorescein fragments and cannot be distinguished. The rate of fluorescein release directly from the intact gel may be described by $[1 - D(t)/D_\infty]/[1 - EP(t)/EP_\infty] = e^{-k_{L1}t}$, where $D(t)/D_\infty$ and $EP(t)/EP_\infty$ are the fractions of drug and erosion probe solubilized at time t , respectively, and k_{L1} is the rate constant for drug release (Fig. S2; Eq. S6). Expectedly, the drug released directly from the gel is slower than the total drug released (Table 1), and becomes more difficult to estimate accurately as the cleavage rate at L1 approaches that for L2 (e.g., when both are MeSO₂-). Rates of cleavage of the tethered fluorescein from the gel via L1 are slightly slower than but—as with gel erosion rates—highly correlated ($R^2 = 0.998$) with those from corresponding soluble PEG-fluorescein conjugates (Table 1). Further, as with PEG conjugates, cleavage of fluorescein tethered to a 4×8 gel by a linker controlled by Mod = CIPhSO₂- was first-order in hydroxide ion with $k_{OH} = 7.6 \times 10^{-4} \text{ M}^{-1}\text{h}^{-1}$ (Fig. S3). The t_{RGEL} was 26 d, which is somewhat slower than the cleavage $t_{1/2}$ of the corresponding PEG conjugate; this is likely due to a substantial fraction of free DBCO end-groups (i.e., >4) remaining on the randomly modified eight-arm PEG, which leads to higher cross-linking density and hence longer t_{RGEL} for complete erosion (Table S1). These experiments clearly show that gels can be designed whereby a tethered drug is completely released at a predictable rate, followed by gel degradation.

Hydrogel-Exenatide Conjugate. The potential utility of the hydrogel system for ultraslow release of a drug was demonstrated with exenatide, a 39-amino acid peptide that is a potent glucagon-like peptide-1 (GLP-1) agonist (22). Because exenatide has a $t_{1/2}$ of ~ 2.5 h in humans and requires twice-daily injections, there is considerable interest in developing long-acting GLP-1 agonists (23); currently, several such agonists—one marketed and several in clinical trials—require once-a-week administration. We attached a β -eliminative $N\alpha$ -azido-linker exenatide (6, Mod = MeSO₂-) (3) to $\sim 25\%$ of the eight CO groups of PEG_{40kDa}(BCN)₈ (13) along with a trace BODIPY erosion probe; the remaining ~ 6 BCN end-groups were reacted with stoichiometric amounts of PEG_{20kDa}(L2-CN-N₃). To decrease the time required to measure drug release and gel erosion, the kinetic study was conducted at pH 8.8, 37 °C (SI Text, Exenatide-PEG Hydrogel). Because the β -eliminative cleavages are first-order in hydroxide ion (above and ref. 3), we could estimate that exenatide is released from the gel with a $t_{1/2}$ of 22 d—similar to the cleavage $t_{1/2}$ of the same linker in a soluble PEG conjugate (Table 1)—and that the gel erodes with a t_{RGEL} of 180 d at pH 7.4, 37 °C (Fig. 4). The longer t_{RGEL} compared with that of the 4×4 gel in Fig. 3A (105 d) is ascribed to this gel having a higher cross-linking density of ~ 4.7 vs. four cross-links per node, which can significantly increase t_{RGEL} (Table S1). We also estimated (Eqs. S6 and S7) that $\sim 88\%$ of the solubilized exenatide was directly released from the gel with a $t_{1/2}$ of 25 d, and $\sim 12\%$ was released as PEG-exenatide fragments from gel erosion—the activity of PEG-exenatide fragments should be minimal because the attachment on exenatide abolishes its binding to the GLP-1 receptor (3). If desired, the rate of erosion—and hence the fraction of released PEG-exenatide fragments—could be further decreased by simply using a β -eliminative linker in the gel cross-links with a slower cleavage rate (Fig. 3A). Studies with 2 mg of exenatide (Bydureon) once weekly have shown that the therapeutic steady-state concentration of exenatide in humans is ~ 70 pM (24). To maintain exenatide concentrations over 70 pM for 1 mo in humans with a hydrogel-exenatide conjugate having a drug release $t_{1/2}$ of ~ 21 d, it would be necessary to attain a maximum serum concentration of ~ 0.18 nM exenatide. Using an exenatide volume of distribution at steady state of ~ 0.4 L/kg (25), we estimate this state could be achieved by an average of $10.4 \mu\text{g}\cdot\text{kg}^{-1}$ exenatide/wk, which, assuming complete bioavailability, could be realized by a monthly s.c. hydrogel containing ~ 3.2 mg exenatide, which compares favorably with 2-mg once-weekly exenatide. Thus, if the in vivo

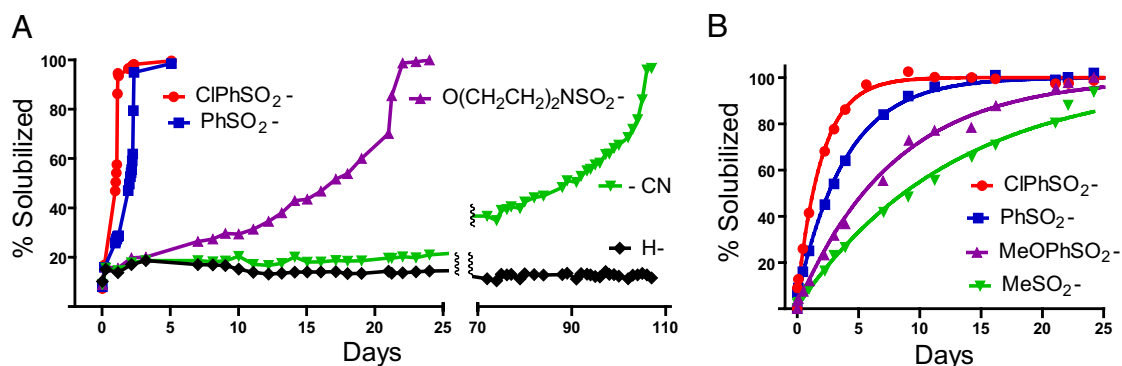


Fig. 3. Degradation of and drug release from hydrogels. (A) Degradation of PEG hydrogels at pH 7.4, 37 °C, as measured by solubilized PEG-fluorescein fragments. Reverse-gelation times using different modulators are ClPhSO₂- = 30 h, PhSO₂- = 55 h, O(CH₂CH₂)₂NSO₂- = 22 d, CN = 105 d; the lines connecting the points are a visual guide in observing the trends of the data. (B) Release of a drug surrogate, aminoacetyl-fluorescein (AAF), using β -eliminative linkers with varying cleavage $t_{1/2}$ values. The lines show the best fit of the data to a first-order rate equation, and SEs were <4.2% of the rate constants (*SI Text, Drug Release from β -Eliminative Linkers*); t_{RGEL} was 630 ± 39 (SD) h ($n = 8$), and observed $t_{1/2}$ values for total drug release using different modulators were ClPhSO₂- = 33 h, PhSO₂- = 65 h, MeOPhSO₂- = 131 h, and MeSO₂- = 212 h.

cleavage rates are similar to the in vitro rates, and if the hydrogel-bound exenatide survives the inflammatory process, an optimized form of this hydrogel should provide an exenatide delivery system that would require only once-a-month s.c. administration.

Discussion

We recently reported an approach to half-life extension of therapeutics that uses a set of self-cleaving linkers to attach drugs to circulating macromolecular carriers (3). The linkers undergo β -eliminative cleavage controlled by an electron-withdrawing modulator to release the native drug with half-lives adjustable from a few hours to over 1 y. These linkers were not susceptible to catalysis by serum enzymes, and showed an excellent correlation between in vitro and in vivo cleavage rates. However, with slowly cleaving linkers, the half-life of the released drug is limited by the elimination of the large conjugate, which is usually no longer than ~1 wk in humans. We therefore sought to use our β -eliminative linkers to tether drugs to noncirculating s.c. implants, where the elimination half-life of a released molecule directly reflects the half-life of the linker used. However, implementation of this approach in vivo also required a carrier that had tunable, long degradation rates, so that the depleted carrier degrades to small, nontoxic components after the drug is released.

In the present work, we have used these β -eliminative linkers to tether drugs or drug surrogates to large-pore PEG hydrogels—analogueous to the near-ideal Tetra-PEG gels (8, 9, 20)—and have

shown they are released over predictable periods. Using similar β -elimination linkers as polymer cross-links, the hydrogels were also tuned to degrade at predictable times over a wide range of ~1–100 d. As shown with Tetra-PEG gels containing ester cross-links, replacing a fraction of the cleavable linkers by stable ones should incrementally decrease the degradation rate of each gel up to at least threefold (26); in this manner, using the reported β -eliminative linkers (3), one could produce hydrogels with finely tunable degradation rates spanning ~1 d to well over a year. Thus, by using one β -eliminative linker to tether a drug to the hydrogel, and another with a longer half-life to control polymer degradation, the cleavage rates can be optimally coordinated to release the drug before the gel undergoes complete degradation.

Cleavage rates of β -eliminative linkers attaching drugs to a gel or cross-linking the gel are highly correlated with their cleavage rates in soluble PEG conjugates, which in turn are tightly correlated with the electron-withdrawing ability of the modulator controlling linker cleavage. As for PEG conjugates, β -eliminative cleavage rates of the linkers are first-order in hydroxide ion. Hence, by raising the pH, rates of reactions that are very slow can be studied over reasonable periods of time; by lowering the pH, the gels or gel components can be stabilized for long periods required for storage. As described, the technology should be applicable to almost any drug—small molecule, peptide, or protein—and any gel sufficiently porous to allow unimpaird drug diffusion—dextran, hyaluronic acid, and others.

Table 1. Summary of β -eliminative linker cleavage half-lives in PEG conjugates and hydrogels

Modulator	PEG*		Hydrogel†	
	Drug release $t_{1/2}$, d	Gel erosion t_{RGEL} , d†	Drug release	
			Total (L1 + L2) $t_{1/2}$, d	From gel (L1) $t_{1/2}$, d
ClPhSO ₂ -	1.5	1.3	1.4	1.5
PhSO ₂ -	3.0	2.3	2.7	3.3
MeOPhSO ₂ -	6.7		5.5	8.0
MeSO ₂ -	19		8.8	21
O(CH ₂ CH ₂) ₂ NSO ₂ -	31	22		
CN	100	105		

*PEG data are from ref. 3.

†Hydrogel gel erosion is from Fig. 3A; total aminoacetyl-fluorescence (AAF) released includes free and attached to PEG fragments as shown in Fig. 3B, and AAF released directly from gel is calculated using Eq. S6 and shown in Fig. S2.

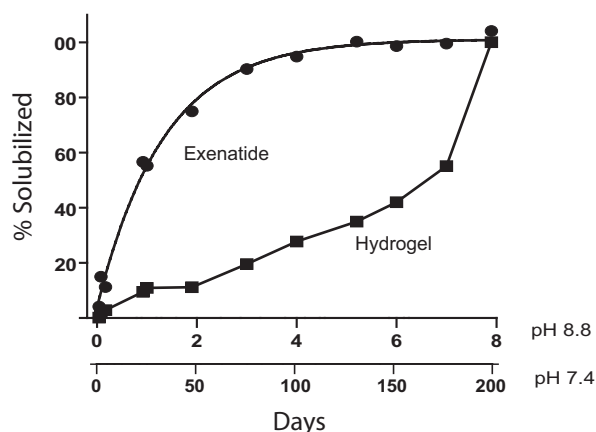


Fig. 4. Exenatide release from and erosion of hydrogel. Data obtained at pH 8.8 [$k = 0.75 \pm 0.057$ (SE) d^{-1}] was estimated at pH 7.4 using $t_{pH7.4} = t_{pH8.8} \times 10^{pH8.8-7.4}$ based on observations that β -eliminative cleavages are first-order in hydroxide ion for drug release (3) and gel degradation (Fig. S1); (—●—) Total exenatide released from gel (free and PEG fragments); (—■—) gel erosion. The line for exenatide release is the best exponential fit of the data, and the lines connecting the gel degradation points are a visual guide in observing the trends of the data.

As an illustration of the potential practical utility of this technology platform, we prepared a PEG hydrogel–exenatide conjugate and showed that the peptide was slowly released with an estimated half-life of ~ 3 wk at pH 7.4; subsequently, the gel degraded with complete dissolution occurring at ~ 6 mo. If these *in vitro* cleavage rates translate *in vivo*, an optimized form of this hydrogel conjugate should provide an exenatide delivery system that would require only once-a-month s.c. administration, and there is no reason to expect this would not also apply to other potent drugs.

The delivery system described here overcomes certain limitations of conventional drug-encapsulating implants. First, covalently cross-linked encapsulating gels depend on limiting pore size for drug retention and gel degradation—usually by hydrolysis of ester linkages within the cross-links—for drug release. Because hydrolysis rates of esters commonly used in polymers are not easily tuned over a wide range, gel network chemistry and structure play the larger role in gel degradation rates, and individually optimized polymers are required for any given drug delivery system (19, 21). In the present work, we desired to avoid changes in polymer network properties that might affect cleavage rates of our β -eliminative linkers. Here, drug release and gel degradation are governed by the small modulators dictating linker cleavage rates, and gel network structure and chemistry are kept relatively constant; these systems do not require individually optimized polymer structures for each drug, and do not require that drug release be dependent on gel degradation rates. Drug-encapsulating systems

can also show initial “burst release” or terminal “drug-dumping” effects at the beginning and end of gel degradation. Here, because the drug is covalently fixed to the hydrogel from the outset, such effects should not occur.

Second, drug encapsulation requires that gel polymerization be performed in the presence of the drug, usually concomitant with formation of injectable micro- or nanoparticles. The process often requires the use of organic solvents incompatible with some drugs—e.g., peptides and proteins—and commonly uses radical-induced polymerization that can lead to irreversible attachment of the drug to the polymer (27). In the present work, the tethering of molecules and gel polymerization reactions both use Cu-free [3 + 2] dipolar cycloaddition chemistry that is orthogonal to biological functional groups and can be performed in aqueous media; this expands the reach of the technology to molecules sensitive to harsh conditions (e.g., peptides and proteins) and should also avoid undesirable irreversible attachment of the drug to the gel (28). Other bioorthogonal coupling reactions, such as Michael addition of thiols (29) or tetrazine cycloadditions with dienophiles (30), should provide similar benefits. Also, because drugs are tethered to components of the hydrogel before cross-linking, it should be possible, but not necessary, to perform the polymerization *in situ* by mixing of filter-sterilized components shortly before injection (30).

Last, ester cross-linked polymers commonly used in encapsulating gels, such as those derived from glycolic or lactic acid (6), produce acid upon hydrolytic degradation that can cause undesirable modifications of peptidic drugs (31). With the present system, the carbamate bonds cleaved upon gel degradation do not produce acid and thus avoid such problems.

There are several relevant extensions of the technology platform. For example, more than one drug could be tethered to a hydrogel by one or more individually tuned linkers to provide a delivery system that releases the drug combination at the same or different rates. Also, absent the tethered drug, smaller mesh-size hydrogels cross-linked by β -eliminative linkers could be used for encapsulation of larger proteins and erosion of the polymer tuned to the desired rate of drug release. Moreover, in addition to s.c. drug delivery, the tunable hydrogels should find utility in other applications, such as regenerative medicine, orthopedic implants, single-shot vaccines, and others.

Materials and Methods

The source of specialized materials is provided, along with their use, in *SI Text*. Detailed synthetic procedures for hydrogel formation are described. *In vitro* kinetic procedures for drug release and hydrogel degradation methodologies are provided. Derivation of equations used to estimate the rate and extent of release of molecules directly from gel are presented in *SI Text, Derivation and Use of Equations*.

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