Tuning hydrogel properties and function using substituent effects†

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The physical properties and function of hydrogels are shown to depend on the substituents present in three novel 1,3,5-triazaadamantane (TAA) cross-linkers. Gel stiffness and degradation rate at varied pHs could be predictably tuned with the cross-linker substituents used to form the gel. Subsequently, protein release from the hydrogel were controlled with chemical structure of the cross-linker.

Hydrogels prepared by cross-linking hydrophilic polymers can resemble a natural extracellular matrix and, thus, have been used for numerous biomedical applications including drug delivery and cell encapsulation.[1] The successful use of hydrogels in these applications very much relies on the ability to tune the hydrogel degradation rate at target tissues which can present different pH environments. Tumor and inflammatory tissues present an acidic environment relative to normal tissue, requiring a gel that degrades under acidic conditions.[2] Other applications involving the implantation of hydrogels in normal tissue require a gel that degrades under neutral conditions.[3] Degradable hydrogels are commonly prepared by incorporating hydrolytically labile ester,[4a] anhydride,[4b] or acetal[4c,d] groups into gel-forming polymers or cross-linkers, however there is still need for new degradable motifs displaying controlled degradation.

Herein we report a method to tune the hydrolysis rates of hydrogels at a given pH by using cross-linking agents containing the 1,3,5-triazaadamantane (TAA) unit. As shown schematically in Fig. 1, these cross-linkers possess a hydrolytically labile TAA unit with three aromatic groups to control hydrolysis rates through substituent effects,[5,6] three acrylamide groups for cross-linking, and a poly(ethylene glycol) chain for water solubility. Reports by several groups have shown that the rate of degradation of acetal-containing hydrogels can be tuned under acidic conditions.[7] In contrast, the TAA group allows the hydrolysis to occur in a highly tunable fashion across a much broader pH range, extending up into the basic regime.

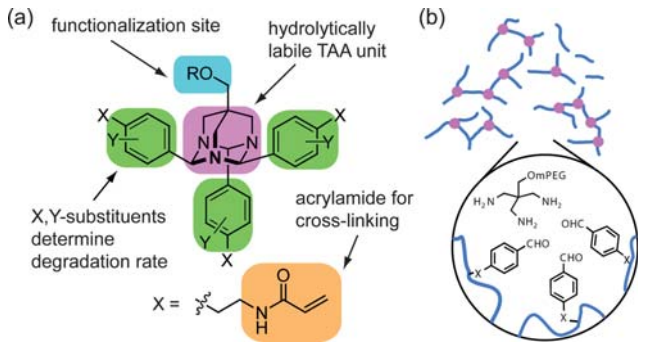
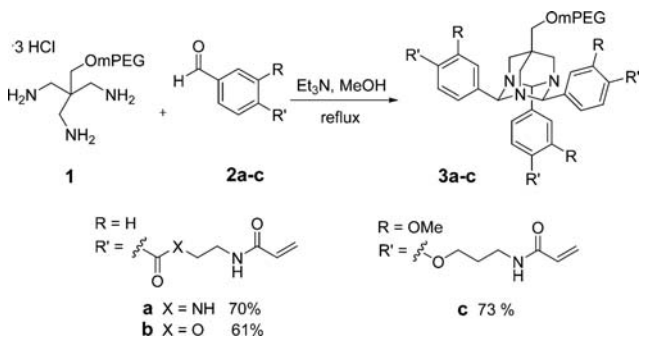


Fig. 1 A schematic showing (a) generalized TAA trivalent cross-linking agent and (b) degradation of TAA unit within gel.

Three TAA cross-linkers were synthesized to present a similar size, architecture, and reactivity but each possesing different aromatic substituent groups (Scheme 1).[8] Thus, TAAs **3a** and **3b** with electron withdrawing groups (amide and ester linkages, respectively) in the 4-position were expected to be comparatively stable in comparison to TAA **3c** with 3,4-alkoxy-substituted (electron rich) aromatic groups. Whereas TAAs **3a** and **3c** were designed to be stable to base, ester-containing TAA **3b** was designed to degrade under both acidic and basic conditions (Fig. S1†).

Polyacrylamide, poly(*N*-isopropylacrylamide), and poly(2-hydroxyethyl acrylate) hydrogels were prepared by *in situ* photochemical or chemical polymerization of the appropriate monomer solution containing TAAs **3a–c**.[8] No gels formed in the absence of TAAs, supporting the role of the TAA functionalized acrylamides as a cross-linker (Fig. 2a *vs.* 2b). The concentration of the TAA determined the mechanical stiffness of the resultant polyacrylamide hydrogels. Thus, the elastic modulus of the hydrogel increased with the ratio of TAA to acrylamide used in the polymerization (Fig. S2a†). The molecular structure of the cross-linker also had an effect on the modulus (Fig. S2b†). Hydrogels cross-linked with TAA **3a** were approximately twice as stiff as those cross-linked with TAA **3b** or **3c** at the same monomer to cross-linker ratio. This result can be explained by the restricted free rotation around the amide bond.

Changes in the aromatic substitution pattern of the TAA units also produced a noticeable change in the hydrogel degradation rates, as



Scheme 1 Synthesis of TAA cross-linkers **3a–c**; mPEG = methoxy poly(ethylene glycol).

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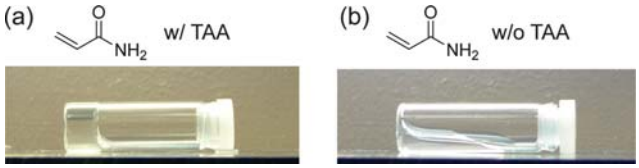

Fig. 2 Acrylamide solution polymerized (a) with and (b) without TAA **3a**. A 1 mol % concentration of TAA was used.

quantified by the increase of hydrogel swelling ratio over time (Fig. 3a). At pH 5.0, gels cross-linked by TAA **3c** degraded >100-fold faster than those cross-linked by TAAs **3a** or **3b** (Fig. 3b). Gels prepared using 1 mol % of TAA **3c** completely degraded within seven hours, whereas analogous gels prepared with TAA **3a** or **3b** slowly swelled over two months. These trends were observed at higher pH values, although the overall degradation rates were lower. At pH 7.4, discs prepared from TAAs **3a** and **3b** swelled very little over the course of 100 days, whereas hydrogels cross-linked by TAA **3c** were fully degraded (Fig. 3c). A further increase of pH to 10 led to degradation of **3b**-derived hydrogels as a result of ester hydrolysis (Fig. 3d).

The hydrogel degradation rates in acidic and neutral conditions were inversely correlated with the Hammett substituent constants (σ), which quantify the electronic contribution of aromatic substituent groups (Fig. 4).[9,10] This result further confirms the role of TAA hydrolysis in the hydrogel degradation under acidic and neutral conditions. In contrast, the degradation rates of hydrogels incubated under basic conditions were independent of σ, suggesting that, in this event, the hydrogel degradation was controlled by base catalyzed cleavage of the ester linkage of gels prepared from **3b** (Fig. S1†).

We further examined the ability of TAA **3a** and **3c**-derived hydrogels to control the release of macromolecular drugs in a sustained manner. Thus, polyacrylamide gels were prepared using a 0.5% (w/v) solution of bovine serum albumin (BSA) and a 1 : 19 ratio of **3a** or **3c** to acrylamide. Incubation of the gel cross-linked by **3c** in an acidic media of pH 5.0, resulted in significant protein release; whereas the BSA release from the gel cross-linked by **3a** was highly limited (Fig. 5a). At pH 7.4, the amount of protein released from the gels cross-linked with **3c** and **3a** was greatly diminished as compared to the acidic conditions due to the decrease in cross-linker hydrolysis (Fig. 5b). The percentage of BSA released after 120 h from gels cross-linked with **3c** under acidic conditions corresponds to the majority of entrapped protein (> 65%), while a significantly lower percentage (10–35%) was released using the other conditions (Fig. S4†). Circular dichroism confirmed that the released BSA had not been denatured from the encapsulation and release processes (Fig. S5†).

Lastly, the cytotoxicity of the hydrogels was evaluated. In this study, hydrogels cross-linked with TAAs **3a–3c** were fully hydrolyzed in strongly acidic media and varying amounts of the degradation products were added to media used for the culture of NIH3T3 fibroblasts. Cells were mostly viable up to 0.2 mg mL^{-1} of degraded hydrogel, but a significant decrease in viability was observed at concentrations above 2 mg mL^{-1}. Compared to polyethyleneimine (PEI), a common polymer known for being toxic, the hydrogel degradation products were relatively biocompatable (Fig. 6). Additionally, polyacrylamide hydrogels prepared from all three cross-linking agents (**3a–c**) resulted in minimal inflammatory response over 4 days *in vivo* when they were implanted into a chick embryo chorioallantoic membrane (Fig. S6†).

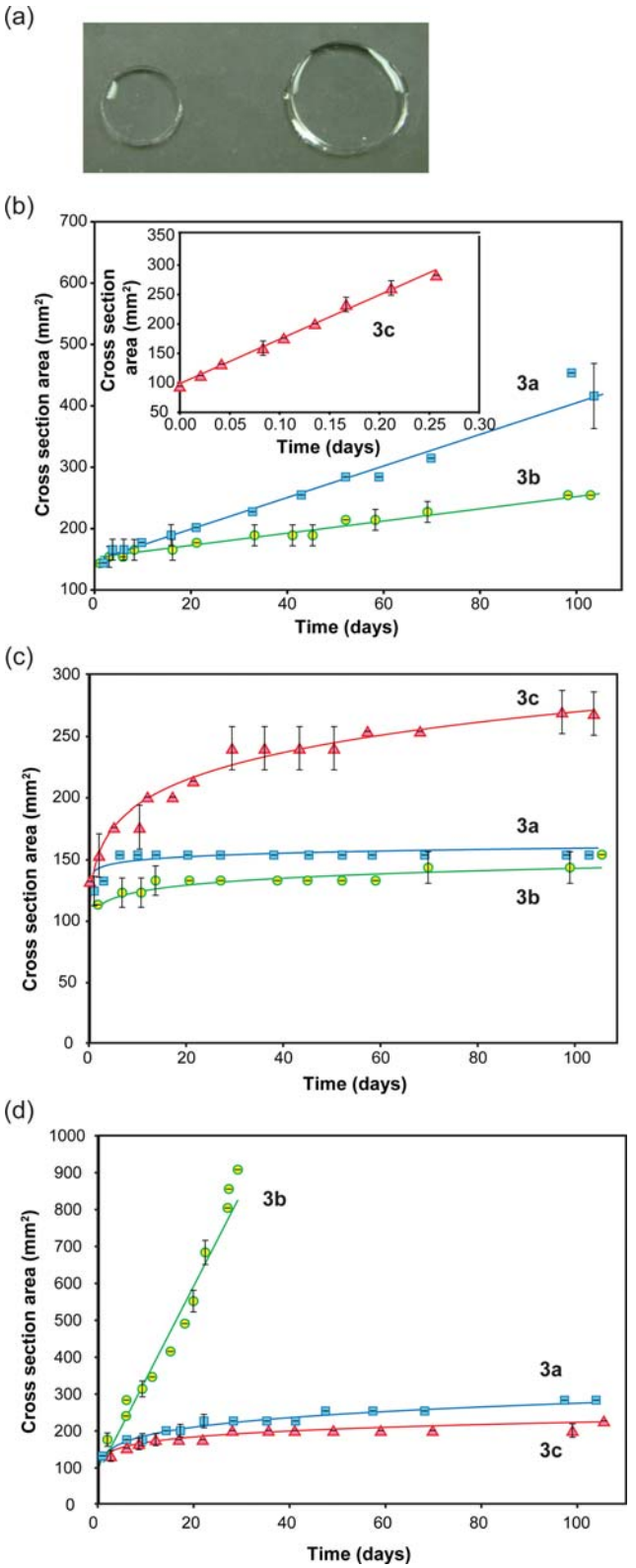

Fig. 3 (a) Images of polyacrylamide hydrogel discs cross-linked with 1 mol % TAA **3c** (left) before and (right) after significant TAA degradation. Changes in the surface area over time of polyacrylamide hydrogels cross-linked by TAAs **3a–c** at (b) pH 5.0, (c) 7.4, and (d) 10.0. Hydrogels were incubated in phosphate buffer saline (PBS) at 37.0 °C. Some data points omitted for clarity; see Fig. S3 for full data.†

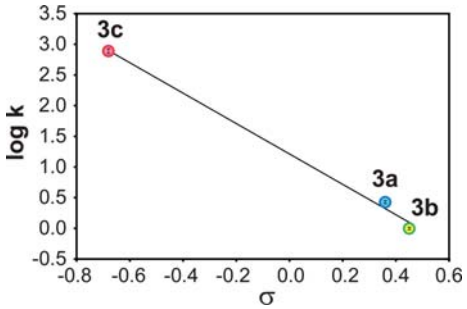

Fig. 4 The correlation of Hammett substituent constants (σ) with the log of hydrogel degradation rates (k)[11] at pH 5.0 of hydrogel discs cross-linked with TAAs **3a**, **3b**, or **3c**.

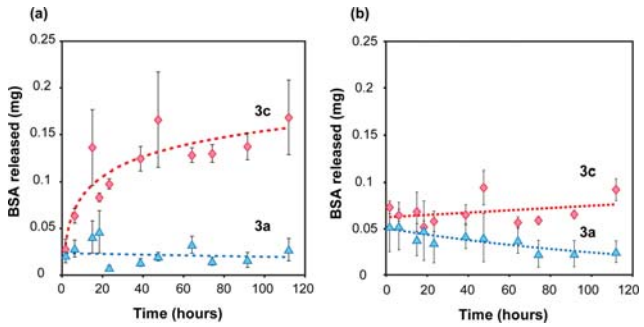

Fig. 5 The release profile of bovine serum albumin (BSA) from polyacrylamide hydrogels cross-linked by TAAs **3a** or **3c**. Hydrogels cross-linked with 5 mol% TAA were incubated in PBS at 37.0 °C at (a) pH 5.0 and (b) pH 7.4.

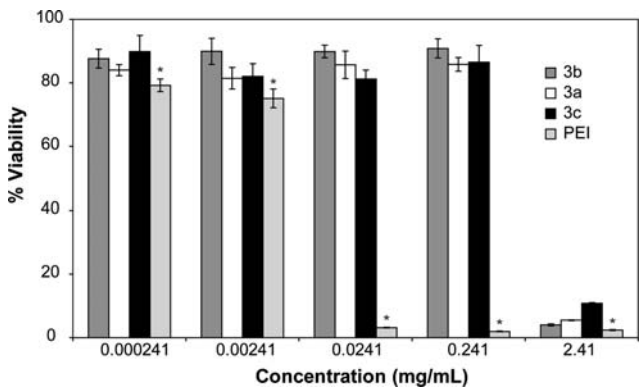

Fig. 6 Cytotoxicity of hydrogel evaluated with viability of NIH3T3 fibroblasts cultured with varying amounts of hydrogel degradation products and branched 25 kDa polyethyleneimine (PEI). Hydrogel degradation products of gels cross-linked with TAAs **3a**, **3b**, and **3c** were statistically significant ($p^* < 0.05$) at all concentrations tested as compared to PEI.

In conclusion, this study describes a novel method to tune hydrogel degradation kinetics using pH-responsive biodegradable cross-linkers. This approach relies on the electronic effect that aromatic substituents have on the hydrolysis rate of the TAA unit. The effects of the cross-linker on the hydrogel degradation rate and subsequent protein release profile in physiological conditions were fully predictable using Hammett constants. We expect that the hydrogel properties and function can be further tuned by controlling the cross-linking density and the polymer concentration in the gel. Such refined control over hydrogel properties and function are desirable for a broad array of applications such as drug delivery that should selectively occur in response to specific pH environments and three dimensional cell culture that should stably last regardless of pH change.[12]

Overall, the TAA cross-linking agents synthesized in this study may prove useful for controlling the properties and function of hydrogels formed from a broader array of monomers[13a] and pre-polymers.[13b] Furthermore, the use of TAAs with multiple reactive acrylamide groups may facilitate the processing of hydrogels in various sophisticated forms including microgel particles.[7a,b,14,15] Ultimately, TAA cross-linkers may help expedite the use of hydrogels as targeted drug delivery devices and tissue engineering scaffolds in clinical settings.

Acknowledgements

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