Synthesis of well-defined hydrogel networks using Click chemistry†

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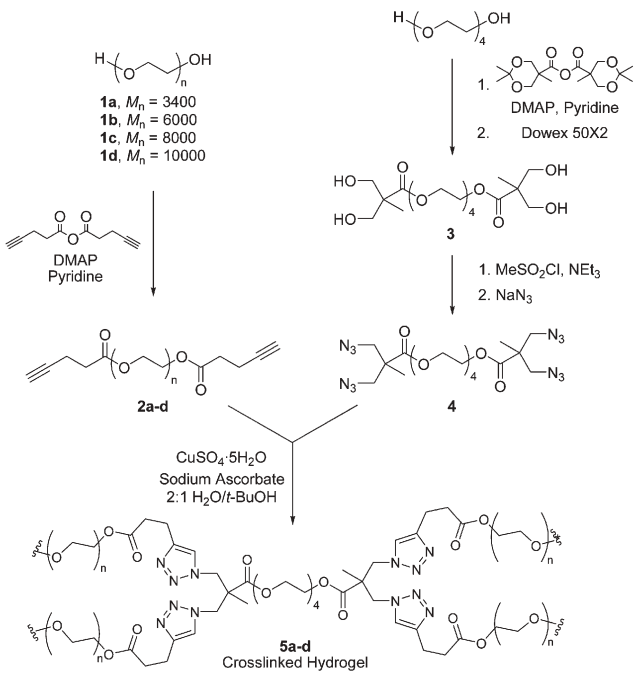
New PEG-based hydrogel materials have been synthesized by Click chemistry and shown to result in well-defined networks having significantly improved mechanical properties; the selectivity of the azide/acetylene coupling reaction also allows for the incorporation of various additives and functional groups leading to chemical tailoring of the hydrogels.

Crosslinked hydrogel materials are a central component in numerous biomedical applications ranging from drug delivery systems to tissue engineering scaffolds.[1] Despite this extensive use, fundamental synthetic studies aimed towards developing new hydrogel materials with improved properties are lacking. Traditional syntheses of crosslinked hydrogel materials involve the photopolymerization of water-soluble vinyl monomers,[2] formation of hydrogel micro- and nanoparticles by solution polymerization,[3] crosslinking of telechelic hydrophilic polymer chains such as poly(ethylene glycol)-diacrylate, *etc.*[4] These strategies demonstrate some of the important chemical requirements for hydrogel synthesis: functional group tolerance, mild conditions, and an efficient, high-yielding reaction. Future hydrogel applications will require significantly improved physical/mechanical properties and increased compatibility with a range of chemically diverse functional groups. Unfortunately, traditional hydrogel synthesis relies upon uncontrolled crosslinking methods, such as radical chemistry. This results in poorly defined materials and increases the difficulty in relating the network structure to the final physical properties of the gel. For these reasons it would be highly desirable to develop alternative chemistries for the formation of crosslinked hydrogel materials which combine the best features of traditional strategies with an increased level of chemical control and diversity.

In devising a new strategy to address these issues, we were guided by two fundamentally important studies. Hubbell demonstrated that cell-responsive hydrogels can be synthesized by crosslinking cysteine-based peptides with vinyl sulfone-functionalized multiarmed poly(ethylene glycol) (PEG) macromers,[5] and similar chemistry was recently used to form hydrogels from PEG macromers and dendritic peptide crosslinkers.[6]

In the second study, Gong demonstrated the critical importance of network structure by preparing interpenetrating double networks with defined crosslink densities from specific monomer sets. These hydrogels displayed extremely high tensile strength and low coefficients of friction.[7] In designing a controlled hydrogel network structure that would be complementary to and extend the orthogonal approach of Hubbell, our attention was drawn to Click chemistry, specifically the copper(I)-catalyzed regiospecific formation of 1,2,3-triazoles from azides and terminal acetylenes.[8] This concept[9] and associated set of reactions have recently garnered a considerable amount of attention due to its complete specificity, quantitative yields, and almost perfect fidelity in the presence of a wide variety of functional groups under physiological conditions.[10] In this report, the Click chemistry concept is utilized for the construction of novel hydrogels with controlled architecture and improved mechanical performance.

PEG was selected as the main structural component for model Click-based hydrogel networks due to its hydrophilicity and biocompatibility. The synthesis of diacetylene-functionalized and tetraazide-functionalized PEG derivatives is shown in Scheme 1.



Scheme 1 Modular approach for hydrogel construction based on Click chemistry and PEG-based building blocks.

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Anhydride chemistry was used in the synthesis of both components, as it has been shown to quantitatively functionalize both polymers and dendrimers.[11] The esterification of hydroxy-terminated PEGs **1a–d** with 4-pentynoic anhydride in the presence of 4-dimethylaminopyridine afforded the desired acetylene-functionalized polymers, **2a–d**, in quantitative yield. The tetrahydroxy derivative **3** was prepared by quantitative esterification of tetraethylene glycol with the anhydride of isopropylidene-2,2-bis(methoxy)propionic acid, followed by mild deprotection with an acidic resin (Dowex 50 × 2). Activation of **3** with mesyl chloride followed by nucleophilic substitution with sodium azide produced tetraazide **4** in 88% overall yield.

To investigate hydrogel formation by copper-catalyzed cycloaddition chemistry, the tetraazide, **4**, was reacted with 2.0 equivalents of PEG diacetylene **2a** (M_n = 3400) at room temperature under aqueous conditions in the presence of copper sulfate and sodium ascorbate as a reducing agent. Hydrogel crosslinking efficiency was found to be influenced by both polymer and catalyst concentration. Eventually a [**2a**] : [**4**] : [Cu] : [ascorbate] ratio of 2 : 1 : 0.4 : 1 was identified that produced swollen hydrogel network **5a** having a gel fraction of 0.95 or greater (Table 1; see supporting information), which is indicative of the highly efficient nature of the Click crosslinking reaction. Under these conditions hydrogel **5a** was formed in less than 30 minutes at room temperature, although reaction times could be reduced to less than one minute when performed under microwave irradiation. Dangling chain-ends and other network defects are largely responsible for limiting the extensibility of hydrogel systems. To demonstrate the efficacy of Click chemistry in preparing model networks, a post-gel modification was performed with both acetylene and azide functionalized chromophores to survey for residual azide/acetylene functionality. For example, gel **5a** was immersed in a water/*tert*-butanol solution of excess *N*-(1-oxopenta-4-ynyl)-5-aminofluorescein with $CuSO_4$ and sodium ascorbate. Any residual azide groups will react with the chromophore, incorporating it into the hydrogel network. After reaction the gel was purified by extensive dialysis and in some cases degradation followed by UV and fluorescence analysis revealed a maximum of 0.2% unreacted functional groups (azide/acetylene values were similar) remained after initial gel formation. This further supports the efficiency of network formation and suggests establishment of a nearly ideal structure.

The controlled nature of the Click coupling reaction also permits the chemical and physical nature of the resulting gel to be finely tuned by small variations in the azide/acetylene ratio. For example, the water content of Click hydrogels based on **2a** and **4** can be increased from 89.0% (for gel **5a**) to 95.4% by changing the molar ratio of **2a** : **4** from 2 : 1 to 3 : 2. This modularity results in an altered gel structure, and the unreacted excess azide or acetylene groups which are not degraded during the reaction can undergo further Click reactions, enabling the introduction of different functional groups such as bioactive molecules into the gel network. However, a slight reduction in the physical properties of the hydrogel is expected. For many biomaterials applications, the presence of copper in the final gels is undesirable, however the level of Cu could be reduced from 0.13 wt% for the crude hydrogel to undetectable levels (ESEM with energy dispersive spectroscopy) by washing with 0.1 M aqueous ethylenediamine tetraacetic acid (EDTA) solution. Finally, it was significant to note the high degree of reproducibility of the results in terms of gel fraction, reaction time and swelling behavior with excellent "shape" memory after many cycles of swelling/deswelling.

Table 1 Physical measurements for PEG-based hydrogels

Gel	Method	PEG M_n (g/mol)	Gel fraction[a]	Swelling degree[b]	Max. true stress (kPa)	Extension to break
5a	Click	3400	0.89	800	680	400%
5b	Click	6000	0.930	1050	1380	1000%
5c	Click	8000	0.950	1050	1660	1250%
5d	Click	10000	0.965	1100	2390	1550%
6a	Photochemical	3400	0.91	600	160	50%
6b	Photochemical	14000	0.89	900	70	150%

[a] Gel fraction = (mass dried gel)/(mass polymer precursors). [b] Swelling degree = (mass swollen gel − mass dried gel)/(mass dried gel) × 100%.

One of the driving forces for constructing hydrogels based on Click chemistry was the belief that controlled network formation would lead to an increase in mechanical strength. In order to investigate the mechanical properties of triazole-PEG based networks, gels **5b–d** were synthesized and compared with traditional PEG hydrogels **6a–b** prepared by free radical photopolymerization of PEG-diacrylate precursors (M_n = 3400 and 14000 kDa, respectively). Swelling data and mechanical measurements for these hydrogels are listed in Table 1. For Click gels **5a–d**, the swelling degree was found to be inversely proportional to the modulus as hydrogel networks incorporating longer PEG chains should have correspondingly larger pores or cavities, resulting in greater water adsorption and a more flexible material. More importantly, both the tensile stress and the tensile strain of Click hydrogel **5a** are significantly enhanced compared to photochemically crosslinked hydrogel **6a**. This enhancement is even more dramatic when the molecular weight of the PEG main chain is increased, as in samples **5b–d**. The extension to break for 10 K Click gel **5d** (1550% extension) is an order of magnitude greater when compared to traditional photo-crosslinked gels based on a higher molecular weight (14 K) PEG, **6b**. Previous studies have shown that the network structure of radically crosslinked gels **6a–b** consists of dense clusters consisting of multiple acrylate groups surrounded by a weakly crosslinked matrix which leads to poor performance.[4b] In addition theoretical studies by Rubinstein[12] predict that the mechanical properties of ideal networks should improve with increasing molecular weight for gels prepared in a swollen state. The dramatically improved properties of the Click gels **5a–d** may therefore be due to the controlled nature of the crosslinking reaction, leading to a more even distribution of crosslink junctions. This ability to modify structure and mechanical properties of hydrogels in a controlled fashion is an important feature for biomedical applications.

Two other critical features of hydrogels that may be enhanced by Click chemistry are environmental stability as well as tolerance to various additives. In both areas the unique attributes of Click chemistry lead to superior performance when compared to traditional approaches for hydrogel formation. The modular nature of this synthetic approach combined with the high degree of stability for the resulting triazole crosslinking group allows tuning of the degradation profile. For the original PEG materials based on the diester, **4**, degradation was found to be dependent on the pH of the aqueous solution. At room temperature, no degradation

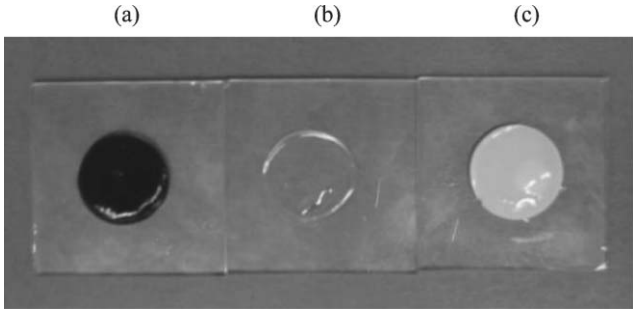
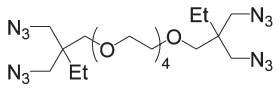


Fig. 1 PEG-based hydrogels formed from **2a** and **4** in the presence of (a) carbon black, (b) 4-phosphonooxy-2,2,6,6-tetramethylpiperidyloxy nitroxide and (c) titanium dioxide nanoparticles.

occurred at pH = 1, slow degradation (greater than 1 month) was observed at pH = 7 and complete degradation achieved at pH = 10 after only 3 hours. In contrast, PEG hydrogels could be prepared from the corresponding diether, **7**, and were found to be stable for greater than 1 month at pH values ranging from 1 to 14.



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Another design advantage of Click chemistry is the tolerant and specific reaction conditions which allows network formation to be performed at room temperature in the presence of a variety of additives which would normally retard or terminate polymerization under traditional radical or photochemical conditions. To examine this feature in detail the preparation of PEG hydrogels based on **2a** and **4** was performed in the presence of carbon black (20 wt%), 4-phosphonooxy-2,2,6,6-tetramethylpiperidyloxy nitroxide (1 wt%) and titanium dioxide nanoparticles (20 wt%) (Fig. 1). The 4-phosphonooxy-2,2,6,6-tetramethylpiperidyloxy nitroxide was chosen as a water soluble radical trap which would terminate either a photochemical or thermal free radical process. Similarly the carbon black and TiO_2 particles have reactive surfaces which can interfere with many polymerization processes while also adsorbing light, rendering the polymerization mixture opaque and therefore incapable of undergoing photopolymerization. Significantly, the gel fraction in each case was essentially the same as for the non-additive case (0.949) and for the nitroxide example the mechanical properties were identical to those for **5a**. For the filled samples the modulus improved significantly (25.2 kPa for carbon black and 33.4 kPa for TiO_2) while the elongation to break decreased less than 5% in both cases. These results demonstrate the advantages of Click chemistry and the ability to employ a wide range of reactive additives without any detrimental effects on network formation.

In summary, new crosslinked, PEG-based hydrogel materials have been synthesized by taking advantage of the fidelity of Click chemistry. The crosslinking in these hydrogels is extremely high, and results in a more ideal structure leading to improved properties when compared to traditional photochemically-crosslinked PEG hydrogels. In addition, unreacted azide and/or acetylene groups can be subsequently functionalized leading to chemical tailoring of the hydrogels and further demonstrating the formation of new, diverse crosslinked materials.

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