J|A|C|S COMMUNICATIONS

Published on Web 10/07/2010

# 'Living' Controlled *in Situ* Gelling Systems: Thiol−Disulfide Exchange Method toward Tailor-Made Biodegradable Hydrogels

De-Cheng Wu,* Xian Jun Loh, Yun-Long Wu, Chee Leng Lay, and Ye Liu*

*Institute of Materials Research and Engineering, A*STAR (Agency for Science, Technology and Research), 3 Research Link, Singapore 117602*

Received July 26, 2010; E-mail: dc-wu@imre.a-star.edu.sg; ye-liu@imre.a-star.edu.sg

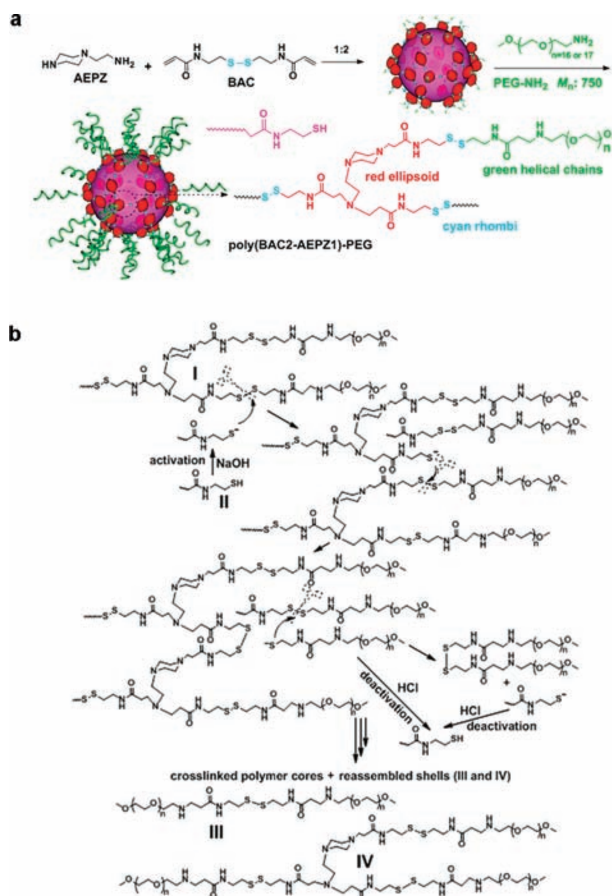
***Abstract:*** A 'living' controlled hydrogel formation method was first reported to create loose and compact *in situ* biodegradable hydrogels. The method executed under mild reaction conditions can conveniently tailor the hydrogel properties, and it has the potential to develop into a powerful tool for the design, synthesis, and self-assembly of novel tailor-made biomaterials and drug delivery systems.

Remarkable advances in living radical polymerizations have enhanced our capability in the development of precisely tailored polymers.[1,2] Nevertheless the preparation of tailor-made polymers remains a grand challenge for the next decade.[3] Hydrogels are unique polymeric materials that have important engineering[4,5] and biorelated[6−10] applications. Diverse approaches have been utilized to produce *in situ* hydrogels with tunable properties using different triggers such as pH,[10,11] temperature,[8,9,12−16] light,[16−19] and targeting biomolecules.[20−23] However, these methods are unable to veritably control the gel properties because of the difficulty in achieving precise control of the extent of cross-linking once the gel formation is initiated. To circumvent this problem, it is greatly desired to design a 'living' controlled *in situ* gelling system with the criteria that the gelation should be able to be activated, controllably terminated and interrupted, and reinitiated by external stimuli whenever needed.

Herein, we demonstrate a facile approach to create a 'living' controlled *in situ* gelling system based on a thiol-disulfide exchange reaction. Thiol-disulfide exchange is a pH-responsive reaction dependent on the p$K_a$'s of the involved thiols, and the reacting species are thiolates.[24] Protonation of thiolates and deprotonation of thiols can be reversibly manipulated by adjusting the system pH, resulting in thiol−disulfide exchange with an 'on/off' function. So, the thiol−disulfide exchange reaction could be utilized to develop the gelling system from a branched precursor with at least three of the disulfide-containing branches cross-linkable through thiol−disulfide exchange. The hydrogel formation is activated, terminated, interrupted, and reactivated by manipulating the pH of the system. The resultant hydrogels are biocompatible, biodegradable, and easily fabricated with desired shapes, sizes, and properties. This 'living' controlled hydrogel formation method can conveniently tailor the hydrogels, and it is a powerful tool for the design, synthesis, and self-assembly of novel tailor-made biomaterials and drug delivery systems.

The model branched polymer was synthesized via one-pot two-step Michael addition polymerization (Scheme 1a).[25] The precursor with terminal vinyl groups was first produced by the reaction of a 1-(2-aminoethyl)piperazine (AEPZ) and a double-molar $N,N'$-bis(acryloyl)cystamine (BAC). Then the vinyl groups were sealed by α-amino-ω-methoxy-poly(ethylene glycol) (PEG, $M_n$ = 750)

***Scheme 1.*** (a) Synthesis of the Branched Poly(BAC2-AEPZ1)-PEG (BAP) and (b) Brief Illustration of Thiol-Disulfide Exchange Reaction Causing the Formation of Cross-Linked Poly(BAC2-AEPZ1) Cores and Release of Reassembled PEG Shells




to yield a core−shell branched polymer, poly(BAC2-AEPZ1)-PEG (BAP), containing a few terminal thiols due to a reduction of disulfide bonds during the preparation and purification procedures (Scheme S1 and Figure S1). Many amines in the core can be protonated to increase the hydrophilicity and stability of the polymer, and the protonation was detected by the shifting downfield of these protons adjacent to amines in $^1$H NMR spectra (Figure S2).

The protonated polymer has high chemical stability during storage and good solubility in water. When a 10 wt % polymer solution (Figure 1A-a) was basified to pH 12, a white loose hydrogel (Figure 1A-b) was formed around 1 h later. The loose hydrogel further shrank into a compact hydrogel (Figure 1A-c) after 1 day of aging. However, there was no further shrinkage in the diameter


10.1021/ja106639c
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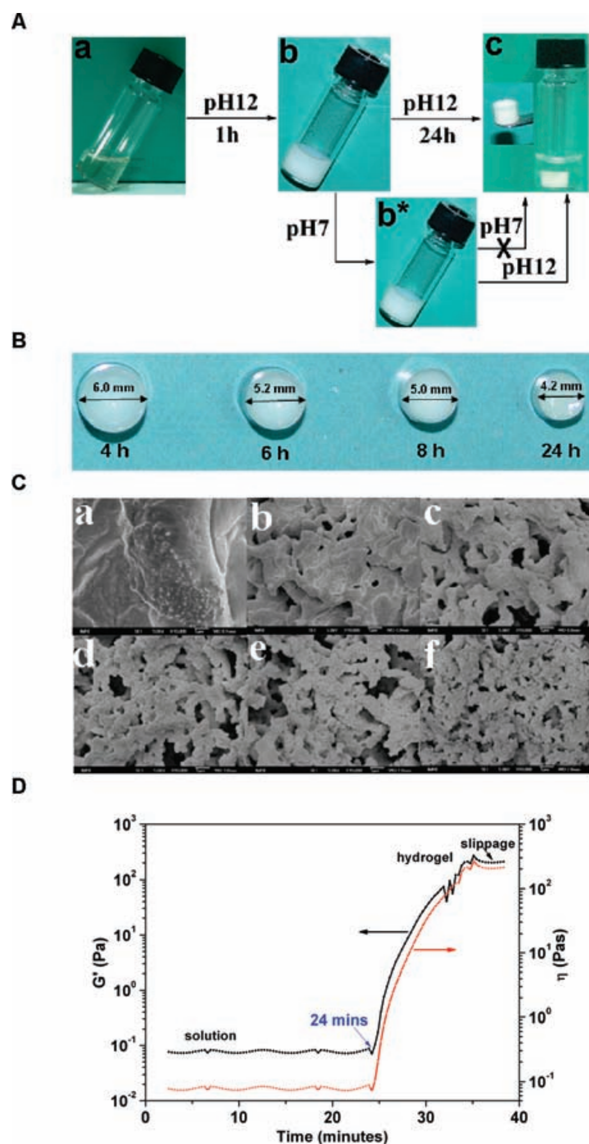


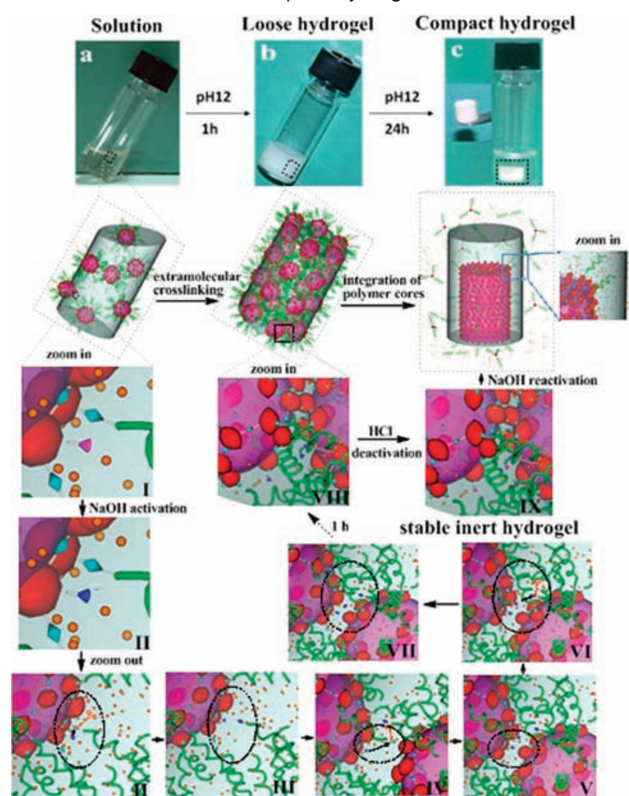

***Figure 1.*** (A) Schematic representation of basification of BAP solution and derived methodology for producing loose and compact hydrogels. (a) 10 wt % BAP solution; (b) loose hydrogel after basification for 1 h; (b*) inert neutralized loose hydrogel after 1 h of cross-linking; and (c) compact hydrogel after basification for 24 h. (B) Variation of hydrogel shrinkage during basification of 10 wt % BAP solution as a function of time. (C) SEM images of the hydrogels obtained after basification for (a) 1, (b) 2, (c) 4, (d) 6, (e) 8, and (f) 24 h. (D) Storage modulus $G'$ and viscosity $\eta$ of 10 wt % BAP solution after basification to pH 12 as a function of time. Oscillatory frequency: 1 rad $s^{-1}$.

of the compact hydrogel when the aging period was extended to 1 week. The transition from the loose to compact hydrogels can be interrupted at any point of time by neutralization and restarted by rebasification (Figure 1A). The neutralized hydrogel (Figure 1A-b*) has good stability and can maintain its shape for over half a year in mild aqueous environments. Figure 1B illustrates the macroscopic shrinkage of the hydrogels during basification and reveals that an increased aging time leads to increased gel shrinkage. The SEM result (Figure 1C) further depicts the hydrogels with significantly different properties and shows that a longer aging time facilitates the formation of a more compact hydrogel with a smaller pore size and denser structure. Therefore, stable hydrogels with customized properties can be easily produced through control of the system pH within a predetermined time. For a clear demonstration of this sol−gel transition, rheological measurement was performed to obtain the storage modulus $G'$ and viscosity $\eta$ (associated with dissipative modulus $G''$). Figure 1D shows that the viscosity of the solution remained constant initially, but after around 24 min, an abrupt increase of both $G'$ and $\eta$ was observed, indicating gelation of the polymer solution. The formation of a stable loose hydrogel was further confirmed by the storage modulus $G'$ being higher than the dissipative modulus $G''$ regardless of a change of oscillatory frequency and a shelf life of 1 month (Figure S3).

The thiol−disulfide exchange reaction plays an important role in triggering *in situ* hydrogel formation. Since the $pK_a$'s of most thiols are 7−11 in aqueous solution, thiols are activated facilitating thiol−disulfide exchange at pH > 11. For the branched polymer, a few thiols should be first converted to thiolates at pH 12, with the thiolates subsequently triggering the disulfide exchange reaction. This leads to cross-linking of the cores and release of the reassembled shells (Scheme 1b). The PEG shell has good solubility in water and can be regarded as a good leaving group, so the driving force was due to the shifting of the equilibrium toward the stable cross-linked products through release of the PEG shells. With a greater extent of exchange, more PEG segments were released and a higher degree of cross-linking was achieved. For verification, solid state NMR and IR spectra (Figures S4 and S5) of the original polymer as well as loose and compact hydrogels after 1 and 24 h of cross-linking were acquired to analyze structure distinction, and the results clearly indicate significantly reduced PEG peaks for the compact hydrogel compared with the original polymer and the loose hydrogel, but the peaks attributed to polymer cores remain the same. Further, the solution of the compact hydrogel was freeze-dried and the recovered products were confirmed to be reassembled PEG shells (Figure S6). Therefore, this evidence supports our hypothesis that the *in situ* formation of the hydrogel can be ascribed to the thiol−disulfide exchange reaction.

The thiol−disulfide exchange mechanism can be applied to interpret the formation and structures of the loose and compact hydrogels and also to demonstrate the hydrogel formation as a 'living' controlled process (Scheme 2). For simplification purposes, one branched polymer unit can be modeled as an isolated core−shell sphere (Scheme 1a). The polymer solution can be regarded as many spheres homogeneously dispersing in water (orange small spheres) (Scheme 2a). In the solution, a few thiols (purple cones in Scheme 2-I) are converted to thiolates (blue cones in Scheme 2-II) under NaOH treatment. The thiolates trigger disulfide exchange reactions (Scheme 2-(II−VII)), linking the two isolated spheres by formation of cross-linked disulfide bonds (blue rhombi) through release of reassembled PEG shells. If the system is aged for 1 h, disulfide exchange leads to the linkage of every isolated sphere by *in situ* buildup of cross-linked disulfide bonds located at the surface of each sphere. The extramolecular cross-links also cause aggregation of the cores forming big hydrophobic domains that prevent light from passing through, so a swollen and loose white hydrogel (Scheme 2b) with a low degree of cross-linking was produced. The hydrogel still contains active thiolates and many untouched PEG shells (Scheme 2-VIII). If it is aged for around 1 day, further disulfide exchange and hydrophobic interactions drive the integration of all the cores to a large hydrophobic core (purple cylinder built from a large number of red ellipsoids linked by cyan and blue rhombi, Scheme 2c), and most of reassembled PEG chains were released into the aqueous phase during disulfide shuffling. The highly hydrophobic cylindrical core expels out a large amount of encapsulated water and shrinks into a compact gel. The nearly depleted number of easy leaving PEG shells and the highly dense and hydrophobic structure of the compact gel that prevents

***Scheme 2.*** Schematic Representation of the Formation and Structures of Loose and Compact Hydrogels[a]




[a] (a) 10 wt % BAP solution, (b) loose hydrogel after 1 h of cross-linking, and (c) compact hydrogel after 24 h of cross-linking.

possible access of the attacking thiolates inhibit further disulfide exchange reaction. Thus no further shrinkage was observed when the aging period was extended from 1 day to 1 week. Meanwhile, thiol−disulfide exchange can be interrupted at any moment by tuning the pH of the system. The thiolates (blue cones in Scheme 2-VIII) of the loose hydrogel can be deactivated to thiols (purple cones in Scheme 2-IX) under neutralization, and the resultant hydrogel has high stability. However, the stable inert hydrogel possesses thiols that can be reactivated whenever needed. This capability demonstrates this hydrogel as having a 'living' capability for differentiation of simply trapped products that are not able to be reactivated. Moreover, active species thiolates exist at the system without disturbance and should be able to further trigger disulfide exchange of another cross-linkable polymer for the formation of 'block' hydrogel system. Currently, we are synthesizing a second disulfide-containing compound for creating a 'block' hydrogel system.

The hydrogel formation method is a 'living' controlled process that is able to produce tailor-made *in situ* hydrogels. The resultant hydrogel is a covalently cross-linked hydrogel without a critical gel concentration. Even 0.5 wt % of a polymer solution can form a suspended hydrogel, but the critical concentration for complete encapsulation of water in the initial solution is around 2.0 wt % for the loose hydrogel after 1 h of cross-linking. In addition, sufficient hydrophilicity of the terminal PEG chain is necessary for hydrogel formation. We investigated the polymers with shorter PEG ($M_n$: 350 or 550) shells and found basification of these two polymer solutions results in solid precipitation instead of hydrogel formation. This could be attributed to cross-linked products without sufficient hydrophilicity to prevent them from aggregation and encapsulating the surrounding water.

The branched polymer is comprised of a biocompatible and biodegradable poly(amido amine) core[26,27] and biocompatible PEG shell and has low toxicity (Figure S7). Since the hydrogel was formed through disulfide shuffling of the polymer cores, the resulting neutralized hydrogels are also biocompatible with low toxicity (Figure S8) and biodegradable (Figure S9). So it is suitable for *in situ* encapsulation of active drugs that can tolerate a relatively high pH. Two model drugs, hydrophilic doxorubicin and hydrophobic paclitaxel, were used to produce drug-loaded hydrogels, and the drug release was investigated. The results (Figure S10) illustrate that tailor-made hydrogels allow fine modulation of drug release to facilitate the preparation of smart drug vectors that are stable in an extracellular medium and decomposable in an intracellular environment to trigger drug release. However, hydrogel formation at a high pH value is not suitable for *in situ* encapsulation of protein and live cells. Hydrogels exist as flowable aqueous solutions but turn into standing gels after pH variation, so they can be easily molded into different sizes and shapes. For example, a cylindrical hydrogel was produced from the solution in a 4 mL cylindrical vial, and a round lamellar hydrogel was obtained from the solution in a round measuring plate during rheological measurement.

In summary, this is the first report of a 'living' controlled hydrogel formation method for tailor-made *in situ* gelling systems. The method utilized a pH-responsive thiol−disulfide exchange reaction to manipulate the hydrogel formation *in situ*. The method can readily tailor hydrogel structures and properties, opening up design possibilities for the *in situ* hydrogels. The method can further be exploited to prepare 'block' hydrogels, micro/nano- hydrogels, hydrogel fibres, and capsules incorporating active agents for drug delivery, tissue engineering, and other biomedical applications.

**Acknowledgment.** We thank the Institute of Materials Research and Engineering (IMRE) for funding this research. We also thank Dr. Maureen Janet for help with light scattering measurement.

**Supporting Information Available:** Detailed procedures and results on the following: polymer synthesis and characterization, hydrogel formation, mechanism and characterization, cytotoxicity assay, and drug loading and release studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA106639C