A Method to Accelerate the Gelation of Disulfide-crosslinked Hydrogels

Xiaoshan Yan, Xiaotong Yang, Xinming Tong** and Yanbin Huang**
Key Laboratory of Advanced Materials (MOE), Department of Chemical Engineering,
Tsinghua University, Beijing 100084, China

Abstract A glutathione-disulfide-ended poly(ethylene glycol) (GSSG-PEG-GSSG) was designed. It is a much more efficient accelerator than glutathione disulfide (GSSG) for the gelation of an 8arm-PEG-SH polymer solution, and the gelation time can be tuned from hours to minutes at the physiological pH and temperature. A mechanism was proposed to explain the different behaviors of the GSSG and GSSG-PEG-GSSG gelation systems. Due to the ever-going thiol-disulfide exchange reaction, the thiol-disulfide hydrogels also showed interesting swelling behavior.

Keywords: Thiol-disulfide exchange; In situ gelation; Glutathione; PEG.

INTRODUCTION

Hydrogels have been widely used for biomedical applications including drug delivery\cite{1-3} and tissue engineering\cite{4-6}, due to their tissue-like water content and easily tunable properties. Among all types of hydrogels, in situ forming hydrogels are injected through a needle in the solution state and gel inside the body via physical association and/or chemical reactions\cite{7}. The in situ gelation avoids the invasive surgical implantation required for the pre-formed hydrogels and is highly desirable for many practical applications\cite{8}.

Hydrogels crosslinked by physical interactions possess intrinsic responsiveness to environmental stimuli, because these crosslinking interactions, such as hydrophobic association, ionic bonding and host-guest complexation, are reversible. However, the downside of these physical hydrogels is the lack of stability and prone to premature erosion or dissolution\cite{9}. In contrast, hydrogels can also be crosslinked by covalent bonds formed by many in situ coupling chemical reactions, such as the Michael addition, hydrazone condensation, oxime formation, alkyne azide 1, 3-dipolar Huisgen cycloaddition and Diels-Alder cycloaddition\cite{7, 10-13}. Compared to the physical hydrogels, most of these covalently crosslinked hydrogels are more stable and better controllable, but the network structure is often 'fixed' once the crosslinking reaction is complete. To combine the advantages of both physical and chemical hydrogels, dynamical covalent linkages such as disulfide bonds, which are capable of undergoing the reversible thiol-disulfide exchange with endogenous and/or exogenous thiol compounds, have emerged as promising candidates to crosslink in situ hydrogels.

While disulfide-crosslinked hydrogels have unique properties and potential in biomedical applications, problems remain in its slow gelation rate (especially for those with low thiol concentrations), unless strong and cytotoxic oxidants such as hydrogen peroxide are added. One way to form disulfide crosslinks in situ is to oxidize free thiol groups of the precursor polymers by the oxygen dissolved in solution, but this reaction is

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** Corresponding authors: Xinming Tong (童新明), E-mail: tongxm@tsinghua.edu.cn
Yanbin Huang (黄延宾), E-mail: yanbin@tsinghua.edu.cn
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usually slow and the gelation time can be longer than 10 h\cite{14}. This slow gelation impedes the practical use, as the polymer components may diffuse away from the injection site before gelation ever occurs. In recent studies, gelation was accelerated by adding disulfide compounds to the thiol-polymer precursor solution, such as cystamine, 2-hydroxyethyl disulfide, 3,3′-dithiodipropionic acid and glutathione disulfide. These disulfide compounds can facilitate disulfide crosslink formation through thiol-disulfide exchange\cite{14,15}. However, there is still a limit to the shortest gelation time achievable with these small molecular disulfide compounds, and the gelation even becomes slower with excessive disulfide compounds\cite{16}.

To overcome the problems with the small molecular disulfide compounds, we here developed a polymer with disulfide groups at both ends, specifically a poly (ethylene glycol) chain with two glutathione disulfides (GSSG) as its end groups (GSSG-PEG-GSSG). Then, we used 8-arm poly(ethylene glycol) with free thiol end groups (8arm-PEG-SH) as the thiol-containing polymer and demonstrated its gelation can be tuned from hours to minutes with the addition of the GSSG-PEG-GSSG polymers (Fig. 1). In addition to the tunable gelation rate, the system uses two types of biocompatible PEG polymers, results in a disulfide-crosslinked in situ PEG hydrogel, and releases the endogenously-abundant glutathione (GSH) as the by-product, all of which make it interesting for biomedical applications.

**EXPERIMENTAL**

**Materials**

Polyethylene glycol (PEG2K, MW 2000 Da), triethylamine (TEA) and calcium hydride (CaH\textsubscript{2}, 90%–95%) were purchased from Alfa Aesar. \textit{p}-Nitrophenyl chloroformate (PNC) was purchased from J&K Chemical. Glutathione disulfide (GSSG) was purchased from Amresco. Toluene, dichloromethane (DCM), diethyl ether, tetrahydrofuran (THF), sodium hydroxide (NaOH) and hydrochloric acid (HCl, 36.5%) were purchased from Beijing Chemical Company. 8arm PEG thiol (8arm-PEG-SH, MW 20 kDa) was purchased from JenKem Technology. DCM was distilled prior to use and PEG2K was dried by azeotropic distillation with toluene. Other chemicals were used as received.

**Synthesis of PEG-\textit{p}-Nitrophenyl Carbonate (PNC-PEG-PNC)^{17} (Fig. 2)**

PEG2K (1.0 g, 0.5 mmol) was dissolved in 20 mL dichloromethane, and then \textit{p}-nitrophenyl chloroformate (PNC, 0.6 g, 3.0 mmol) was added to the solution with magnetic stirring. Triethylamine (TEA, 0.28 mL, 2.0 mmol) was added dropwise into the solution, and then the mixture was stirred for 36 h at room temperature. After the reaction, most of DCM was removed with rotary evaporator and then THF (5 mL) was added to precipitate the unreacted PNC and TEA salts. After removal of the insoluble solid by filtration, the clear solution was added dropwise into ice-cold ethyl ether to precipitate out the product. White solid powder was obtained and washed three times with 500 mL ice-cold ethyl ether and dried in vacuum (yield about 80%).