The Strain-Promoted Alkyne-Azide Cycloaddition Reaction as a Cross-Linker for Making Poly(Ethylene Glycol) Hydrogels for Cell Therapy Applications

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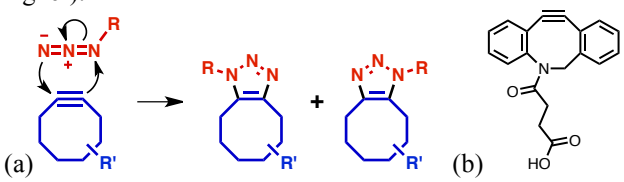
Statement of Purpose: Hydrogels have great potential for cell therapy, as they closely resemble the natural extra-cellular matrix in the body, as well as protecting the therapeutic cells from the host's immune system. Hydrogels made with synthetic polymers pose an advantage over natural polymers, largely owing to the increased amount of structural control, purity and reproducibility. Poly(ethylene glycol) (PEG) is a suitable material as it is hydrophilic and does not elicit an immune response. To covalently cross-link the polymer, the Strain-Promoted Alkyne-Azide Cycloaddition (SPAAC) reaction can occur (Scheme 1a) at the ends of azide- and alkyne-functionalized PEG chains. This reaction is fast, efficient, does not produce any by-products and does not require a catalyst. Here we describe the synthesis of the strained cyclooctyne, aza-dibenzocyclooctyne (DIBAC) (Scheme 1b), attachment of DIBAC to PEG and the reaction of the resulting PEG-alkyne with a synthesized PEG-azide. Hydrogels are formed within minutes at room temperature above certain concentrations (5% w/v or higher).



Scheme 1. (a) The Strain-Promoted Alkyne-Azide Cycloaddition reaction and (b) the specific cyclooctyne being used, aza-dibenzocyclooctyne (DIBAC)

Methods: Aza-dibenzocyclooctyne (DIBAC) was synthesized as previously reported using a modified Popik synthesis [1]. DIBAC was attached to both ends of a 10,000 MW PEG chain via an esterification reaction to make $PEG_{10k}(DIBAC)_2$. The azide monomer, containing two azide groups, was synthesized in two steps as previously reported and ring-opened onto either end of a 10,000 MW PEG chain [2]. The reaction enables control over the number of azide moieties on the polymer chain. PEG hydrogels were made by first pipetting a known amount of azide-functionalized polymer and alkyne-functionalized polymer into a 2mL screw-cap vial. The clear solution was then manually mixed at room temperature with a metal spatula until the mixture became noticeably viscous. In each case of hydrogel formation, the ratio of $PEG_{10k}(N_3)_8$ to $PEG_{10k}(DIBAC)_2$ was 4:1 respectively such that there was a 1:1 ratio of azide to alkyne functionality.

Results: Improving the synthesis of the cyclooctyne, DIBAC, was the key component in being able to create hydrogels efficiently. We were able to substantially increase the overall yield from 21%, as previously reported in the literature, to 67% [3]. We were able to reduce purification to only one chromatographic step, making this procedure possible to complete in two to three laboratory days. The time required for hydrogel formation changed upon varying the concentration of polymer in water. At 20% w/v of both initial polymers, $PEG_{10k}(N_3)_8$ and $PEG_{10k}(DIBAC)_2$, a dense hydrogel was formed within 30 seconds, and was not easily deformed (Figure 1a). Lowering the polymer concentration to 10% w/v of both polymers, a hydrogel formed in 1.5 minutes, resulting in a more flexible hydrogel (Figure 1b). At a concentration of 5% w/v, hydrogel formation occurred within 4.5 minutes (Figure 1c). Hydrogel formation did not occur at 2.5% w/v concentration, even after an extended period of time (Figure 1d). This demonstrates that there is a minimum concentration required for hydrogel formation with the given PEG system. The optimal concentration was determined to be 10% w/v, based on this preliminary bulk gel analysis.

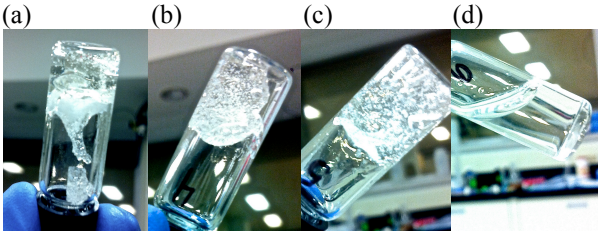


Figure 1. PEG Hydrogels. Formed by Manually Mixing aqueous solutions of $PEG_{10k}(N_3)_8$ and $PEG_{10k}(DIBAC)_2$ in a stoichiometric ratio of 4:1, respectively. Hydrogels were all made at different concentrations of polymer: (a) 20% w/v, (b) 10% w/v, (c) 5% w/v, (d) 2.5% w/v

Conclusions: The Strain-Promoted Alkyne-Azide Cycloaddition (SPAAC) Reaction is an effective way to make PEG hydrogels fast and efficiently without the need for organic solvent, catalysts, UV light, high temperature or high pressure. No other reagents are necessary for gelation to occur, other than the two PEG chains that are functionalized with alkynes and azides for cross-linking. The system described has the potential to be very useful for cell therapy applications. The desired therapeutic cells could be added to one of the polymer solutions prior to gelation. Once the two polymer solutions are mixed, gelation can occur at physiological temperatures without any damage occurring to the cells. Future work includes cell studies specifically looking at cell viability in the hydrogel system (cell-matrix and cell-cell interactions), as well as animal studies looking at the amount of swelling occurring due to the presence of the hydrogel *in vivo* (host-matrix interactions).

References:

[1] Chadwick, R. C. and Van Gyzen, S., et al. Synthesis. 2014;46:A-I. [2] Xu, J., et al. Macromolecules. 2011;44:2660-2667. [3] Campbell-Verduyn, L. S., et al. Angew. Chem. Int. Ed. 2011;50:11117-11120.