Diels-Alder Crosslinked Hydrogels

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Introduction:

Encapsulation of cells within semi-permeable polymer hydrogels is a promising approach for the treatment of hormone or enzyme-deficiency disorders such as diabetes and Tay-Sachs Syndrome. The hydrogels, designed to provide protection from the immune system, are electrostatically or covalently crosslinked, both to enhance strength and to help control the permeability. Reactive polymers that are able to form covalent crosslinks, while not adversely affecting the encapsulated cells or the host, are needed for these applications.

This work examines the Diels-Alder reaction, a selective [4 + 2] cycloaddition between a diene (furan) and dienophile (maleimide), as a potential crosslinking reaction. Poly(methyl vinyl ether-*alt*-maleic anhydride), which has shown promising results as a biomaterial,[1] was reacted with furfurylamine or *N*-(2-aminoethyl)maleimide, before hydrolyzing the remaining anhydride groups, to form poly(methyl vinyl ether-*alt*-maleic acid) functionalized with either furfuryl or maleimide groups (PMM-FFA and PMM-MAL). This presentation will describe the preparation and characterization of PMM-FFA and PMM-MAL, as well as the preliminary results on the gelation of these polymers both in solution and while entrapped within spherical calcium alginate beads.

Materials and Methods:

Poly(methyl vinyl ether-*alt*-maleic anhydride) (80 kDa, Aldrich) was functionalized by reaction with furfurylamine (Aldrich) or *N*-(2-aminoethyl)maleimide (Santa Cruz Biotechnology Inc.) in acetonitrile in the presence of triethylamine. Residual anhydride groups were hydrolyzed by the addition of water before the polymers were purified by dialysis. Fluorescent versions of the polymers were prepared by including small amounts (1 mol%) of fluoresceinamine (Aldrich) or rhodamine cadaverine (Invitrogen) during the functionalization. Bulk hydrogels composed of PMM-FFA and PMM-MAL were prepared by mixing 5-10 w/v% solutions of each polymer to give a total polymer concentration of 5-10 w/v%. The effects of various gelation conditions, including temperature, pH and ionic strength were examined. The rate and extent of the Diels-Alder reaction were quantified using NMR spectroscopy. In the case of polymer-polymer reactions, high-resolution Magic Angle Spinning (HR-MAS) NMR spectroscopy was used to probe the viscous liquids or gels. Rheology was used to determine the gelation time and the mechanical properties of the gel while swelling studies were used to test the stability and degree of crosslinking of the gels.

Matrix beads were formed by gelation of droplets of sodium alginate (1 w/v%) containing various concentrations of PMM-FFA and PMM-MAL, in a calcium chloride (1.1 w/v%) gelling bath. Fluorescence microscopy was used to determine the efficiency of entrapment and distribution of PMM-FFA and PMM-MAL within the bead core. In-diffusion of fluorescently-labelled dextrans of various molecular weights was used to probe the pore size of the beads. Sodium citrate, a calcium chelator, was used to liquefy the alginate inside the beads following

various curing times to determine whether a covalently crosslinked network had been formed by PMM-FFA and PMM-MAL.

Results and Discussion:

PMM-FFA and PMM-MAL with degrees of functionalization ranging from 5 to 40% were prepared as shown by NMR analysis. A model reaction between PMM-FFA and ethylmaleimide, a small-molecule, showed that the Diels-Alder reaction was selective (no side reactions) and efficient where all of the furan groups had reacted within a few hours at 20°C for a solution containing 1.7% PMM-FFA (42% functionalized) and 80 mM (2.3-fold excess) ethylmaleimide.

Bulk gelation studies conducted with mixtures of 10% functionalized PMM-FFA and PMM-MAL at a 1:1 furan/maleimide ratio suggested that the crosslinking reaction was much slower or less efficient than the model reaction. Total polymer concentrations >5% were required to get gelation which took anywhere from a few hours (10% total polymer) to days (5% polymer). Electrostatic and steric repulsion between the two anionic polymers was likely responsible for the slow reaction. The rate of gelation could be increased by using a) polymers with higher degrees of functionalization, b) lower pH, c) higher ionic strength or d) higher temperatures. It was also seen that over a period of 60 days, the gels remained intact, indicating a high level of permanency.

Matrix beads were prepared by extruding a 1% alginate solution containing PMM-FFA10*r* and PMM-MAL10*f* into a calcium chloride gelling bath. In all cases, the beads were of similar size and shape as those formed using alginate alone. For example, the beads formed from 1% alginate and 0.5% PMM-FFA10*r* and 0.5% PMM-MAL10*f* were spherical with smooth surfaces and an average diameter of 550 ± 40 μm. UV/Vis analysis of the solutions used to gel and wash the beads indicated loss of about 5 to 10% of each of the labelled polymers during gelation and washing. This relatively low polymer loss indicates that the polymers are effectively trapped by the forming calcium alginate gel. Confocal fluorescence microscopy of the matrix beads indicated good distribution of both reactive polymers within the core of the beads. Subsequent citrate challenges of the matrix beads demonstrated that the beads remain intact upon liquefaction of the alginate, provided sufficient time for covalent crosslinking. In-diffusion studies of fluorescently-labelled dextran showed that 10kDa dextran is able to rapidly diffuse into the beads, reaching equilibrium within about 10 minutes. 70kDa dextran diffuses in at a significantly lower rate. The 250 and 500 kDa dextrans are partially excluded from the beads, indicating that these Alg-PMM-FFA/PMM-MAL matrix beads show differential permeability for lower and higher-MW species.

The formation of covalently crosslinked networks by Diels-Alder coupling between two diene and dienophile-modified polyanions is demonstrated both in dilute solution, and within calcium alginate templates. The resulting gels retain their shape following calcium extraction, and offer interesting building blocks for long-term cell encapsulation.

References:

1. Gardner, C. M.; Burke, N. A. D.; Stöver, H. D. H. *Langmuir* **2010**, *26*, 4916-4924.