Injectable Hydrogel based on Supramolecular Inclusion Complexes between Alginate-graft-Cyclodextrin and Polypropylene Glycol

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Statement of Purpose: The goal of this study was to design a novel injectable hydrogel based on the formation of supramolecular inclusion complexes between a host polymer, alginate-graft-cyclodextrin (Alg-g-CD), and a guest polymer, polypropylene glycol (PPG). The synthesis of Alg-g-CD was performed in two ways, either in an aqueous or organic solvent, providing a versatile strategy to form the hydrogel. Gelation occurred after mixing Alg-g-CD and PPG solutions in the absence of crosslinking agents and external energy activation, which is promising for biomedical applications, such as controlled drug and cell delivery and *in vitro* 3D cell culture.

Methods: The reaction scheme for Alg-g-CD is presented in Figure 1. Acentontrile dissolved ρ-Toluenesulfonyl (TosCl) chloride was added to β-CD aqueous solution at 4ºC for 2 h to form β-CD-TosCl. A 3:1 equivalent of sodium hydroxide was added drop-wise and stirred for 30 min at RT. The product was washed with ice water and acetone and dried under vacuum. For the reaction in organic solvent, hexanediamine (HDA) was added to β-CD-TosCl in dimethylformamide to react for 18 h at 80ºC. The crude product was precipitated with cold acetone, washed with diethyl ether, and dried under vacuum to afford β-CD-HAD. β-CD-HDA was coupled with alginate-tetrabutylammonium (Alg-TBA) via amidation.[1,2] For the aqueous-based chemistry, β-CD-TosCl was added to ethylenediamine (EDA) under N_2 at 60ºC and mixed for 12 h. After the reaction, ethanol was added to precipitate β-CD-EDA. Alg-g-CD was synthesized from alginate and β-CD-EDA via carbodiimidechemistry.[2]

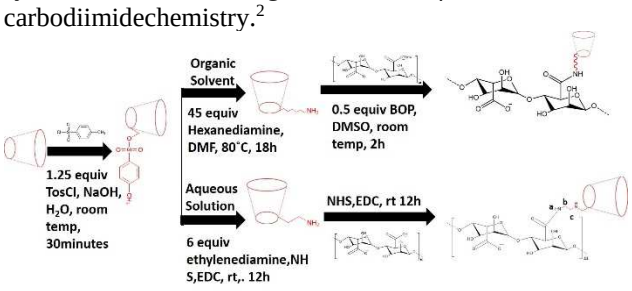

Figure 1 Two approaches to synthesize the guest copolymer Alg-g-CD.

To prepare the hydrogel, Alg-g-CD was dissolved in phosphate buffered saline to make a 1% (w/v) solution. An equal volume of PPG (either MW=400 or MW=1,000) was added to the Alg-g-CD solution and mixed vigorously. Solutions were placed in microcentrifuge tubes and inverted frequently until gelation was complete. All of the hydrogels formed after standing at RT for > 5 min. As a positive control, pure CD was added to PPG solutions.

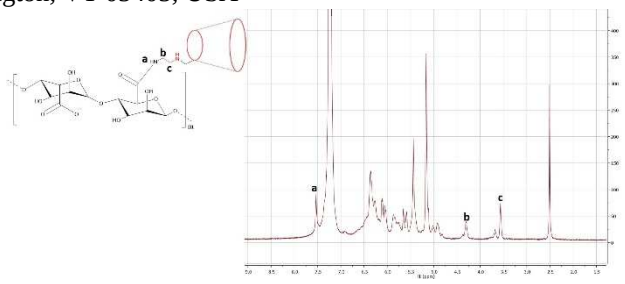

Figure 2 ^{1}H-NMR spectrum for Alg-g-CD formed via aqueous chemistry.

Results and Discussion: The synthesis of Alg-g-CD was verified by ^{1}H-NMR (Figure 2). The formed alginate-based hydrogels are shown in Figure 3. Hydrogels were more likely to form using PPG with a lower molecular weight (MW=400) and Alg-g-CD synthesized using either organic solvents or aqueous-based reactions. PPG with a higher molecular weight (MW=1,000) was only able to form a hydrogel with Alg-g-CD synthesized in aqueous solution. In addition, pure CD formed hydrogels with PPG of different molecular weights; however, the gelation time was longer compared to the alginate-based hydrogels.

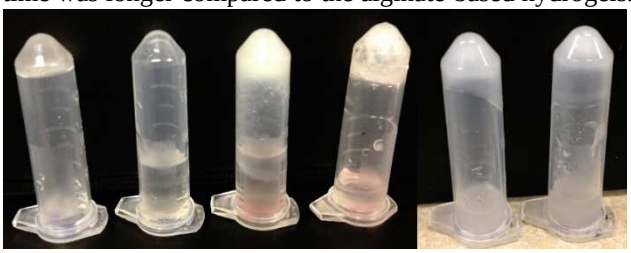

Figure 3 Overview of hydrogel formation. From left to right:
a. Alg-g-CD synthesis in organic solvent + PPG(MW 400);
b. Alg-g-CD synthesis in organic solvent + PPG(MW 1,000);
c. Alg-g-CD synthesis in water+ PPG(MW 400);
d. Alg-g-CD synthesis in water+ PPG(MW 1,000);
e. CD+ PPG(MW 400); f. CD+ PPG(MW 1,000).

Conclusion: An injectable hydrogel was successfully synthesized by utilizing the physical interactions between the host polymer Alg-g-CD and its guest polymer PPG. By conjugating β-CD onto the alginate polysaccharide backbone, we were able to increase the stiffness of hydrogel while decreasing the cytotoxicity. The rheological properties of the hydrogel will be examined at 25 and 37ºC by oscillatory time sweeps at various frequencies (0.01, 1.0, 10 or 100Hz; 1% strain). Cytocompatibility experiments with human primary cells are ongoing.

References: 1. Rodell *et.al*, Biomacromolecules, 2013; 2. Izawa *et al.* Journal of Materials Chemistry B, 2013.