Synthesis of novel low molecular weight alginate-poly(methyl methacrylate) hybrid material using living radical polymerization for controlled drug delivery.

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INTRODUCTION AND OBJECTIVE

Alginate is a biocompatible anionic biopolymer, capable of crosslinking with divalent cations (Ca^{2+}) and entrapping water thus forming a hydrogel. It has been employed in encapsulation and delivery of peptide based drugs, such as insulin, which are susceptible to gastric pH and enzymatic digestion. Native alginate hydrogel has high water solubility and permeability, causing excessive swelling and premature drug release. Attempts have been made to functionalize alginate in order to improve its hydrogel properties and a drug release profile.

Because of high molecular weight of natural alginate, it has difficulty being cleared from the body during intravenous and oral administration. Several previous studies report successful molecular weight reduction of alginate using oxidizing agents such as potassium persulfate and hydrogen peroxide that reduces the molecule size below the renal clearance and also reduces solution viscosity and functional group accessibility for easier chemical modifications. We implement this strategy as a first step in alginate modification. Alginate is usually grafted with various synthetic polymers in order to increase a hydrophobic character for prolonged drug release. In this study we apply a new polymerization technique, living radical polymerization (LRP) which was previously successful in grafting synthetic polymers from other biopolymers such as cellulose and chitosan. LRP, compare to traditional polymerization processes offers a more controlled polymerization process resulting in higher conversions, longer chain lengths and low polydispersity. A preparation process for a successful LRP involved improving alginate solubility in organic solvents and attachment of LRP initiator onto alginate backbone.

MATERIALS AND METHODS

Synthesis of Low Molecular Weight Alginate (LMWA)

1.5% solution of sodium alginate in water or PBS was treated with KPS or H_2O_2/Ascorbic Acid at 80C for up to 6 hours. Time samples were precipitated and washed with alcohol and molecular weight determined by a Malvern ZetaSizer. Presence of LMWA was confirmed by H-NMR (Bruker 400Hz).

Modification of LMWA's solubility in organic solvents

LMWA was acidified in 0.6M HCl/EtOH, filtered, resuspended in water and neutralized with tetrabutylammonium hydroxide (TBAOH). Solution was lyophilized and LMWA-TBA was confirmed by H-NMR.

Synthesis of LMWA-Br macroinitiator

Bromoisobutyric acid was activated with various coupling agents and then added to LMWA-TBA in DMSO/TBAF or DMF/TBAF for a 12 hour esterification reaction. Structure of LMWA-Br was confirmed by H-NMR and degree of substitution (DS) was calculated by integration of H-NMR peaks.

Living Radical Polymerization of methyl methacrylate (MMA) from LMWA-Br macroinitiator

Polymerizations were conducted in water or D_2O with ligand:initiator molar ratios of 1:5. Monomer (MMA) amounts were varied. Copper wire was used as a LRP catalyst. With all components dissolved in water, the solution was deoxygenated and polymerization was started by leaving the solution at 25C. Samples were taken every hour for D_2O reaction. The final product of reaction in water was precipitated and thoroughly washed in acetonitrile and alcohol. pMMA was confirmed by H-NMR.

RESULTS AND DISCUSSION

Figure 1 shows an example of a kinetic degradation experiment. Over time, MW reduction of alginate from 350 kDa down to 56kDa in the presence of KPS was confirmed by DLS. Other degradation experiments involving higher oxidizer concentration and longer reaction time produced MW as low as 20kDa.

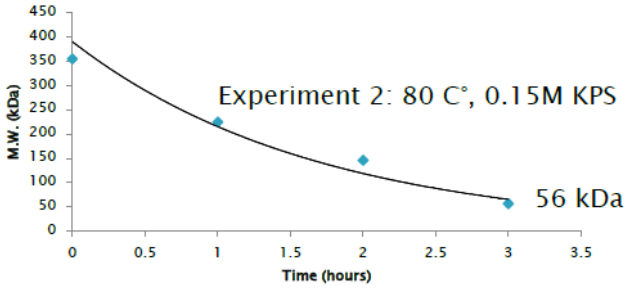


Figure 1. Partial degradation of sodium alginate with KPS to produce LMWA of 56kDa

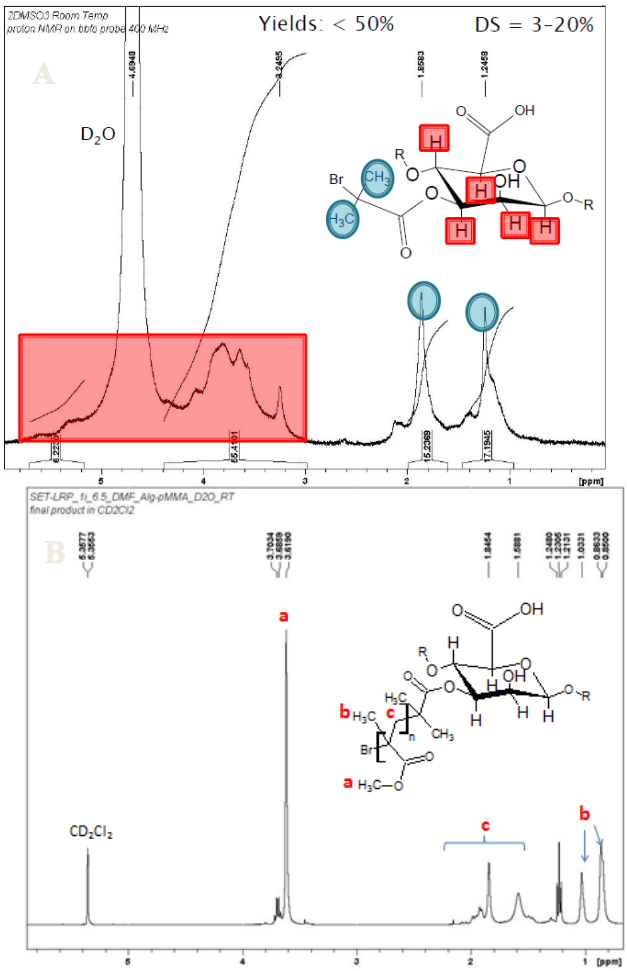
Chemical modifications with alginate are limited due to its poor solubility in organic solvent systems. In our case, functionalization of alginate macroinitiator is a water sensitive reaction and had to be done in an organic polar aprotic solvent such as DMSO, DMF or THF. Therefore, tuning alginate's solubility for such reaction was a key step. Alginate-TBA salt was now soluble in DMSO and DMF containing 1% (w/v) of

TBAF. Such solubility improvement allowed us to proceed to a water sensitive esterification reaction.

Successful attachment of a bromoisobutyryl group onto alginate was confirmed by the H-NMR of the purified product from the esterification reaction.

Figure 2A shows two new peaks between 1-2ppm which are characteristic of methyl Hs on bromoisobutyryl moiety. Substitution varied from 3 to 20% and was inversely proportional to the product yield due to increased solubility in alcohols used in product washing.

LRP of MMA from LMWA was confirmed by H-NMR and increased viscosity over time. H-NMR of heavily grafted alginate shows a well-defined structure of pMMA (Figure 2B), while lower amount of MMA allows seeing both alginate and pMMA Hs (Figure 2C). Having showed successful LRP from alginate we are now engineering a wide range of grafted alginates that will differ in alginate MW, and degree of substitution that will dictate the number of initiating sites per alginate molecule, and finally the length of the synthetic grafts. We are predicting that these grafted LMW alginates will express different physiochemical properties and drug loading capabilities. LMWA molecules containing only few hydrophobic grafts (pMMA) might be able to self-assemble and entrap the drug within their hydrophobic nuclei; while more substituted alginates will be able to load the drug via Ca2+ cross-linking.



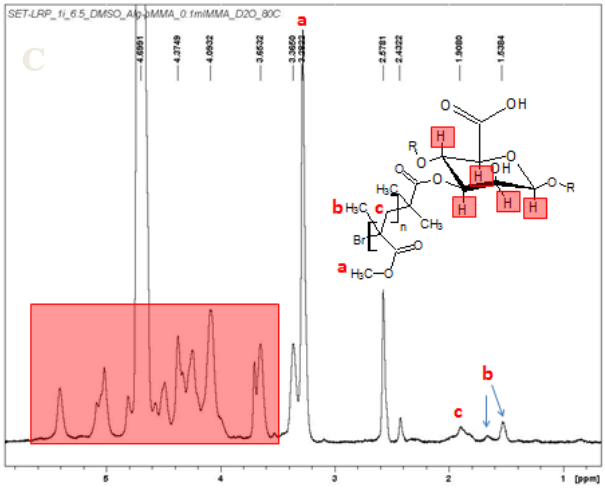


Figure 2. H-NMR spectrum of LMWA-Br macroinitiator (A) and LMWA-pMMA products with monomer to initiator ratio: 200:1(B) and 20:1(C)

CONCLUSION

A new type of alginate based biosynthetic material was prepared by first reducing the molecular weight of native alginate, functionalizing alginate backbone for living radical polymerization and finally grafting synthetic polymer to produce low molecular weight hybrid molecules with an increased hydrophobic character with a potential for improved controlled drug delivery applications.

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