Novel Alginate Based Amphiphilic Drug-Conjugated Graft Copolymers for Controlled Co-Delivery of Antitumor Agents: Current Progress in Polymer Synthesis

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INTRODUCTION

Amphiphilic polymers have been widely used in encapsulation and delivery of hydrophobic drugs due to their ability to self-assemble in aqueous solution into micelles, polymersomes, vesicles, nanotubes, etc. An amphiphilic polymer has distinctive water soluble and water insoluble regions in a molecule. Alginate, a naturally found electrolyte polysaccharide, was chosen to be a hydrophilic block due to its biocompatibility. Less water soluble or hydrophobic polymers were synthesized using single electron transfer living radical polymerization (SET-LRP), a relatively recent polymerization technique with greater control over molecular weight and polydispersity compared to traditional free radical polymerization. Other polysaccharides have been previously modified with polymers via LRP methods (Voepel 2011). Because alginate, in its natural form, is a high molecular weight polymer, it was partially degraded to produce low molecular weight fragments (Xiaoxia 2010) and modified to be soluble in organic solvents for further reactions (Pawar 2011).
We are currently working on two different synthetic approaches to make alginate based amphiphilic polymers. First one is a 'graft from' strategy, where the hydrophilic polymer is grown directly from alginate. Second is a 'graft onto' approach, where a polymer is synthesized first and then clicked onto alginate.

Recent advances in drug delivery show that self-assembled vesicles with drugs chemically conjugated to the polymer produce more controlled and sustained drug release (Wenchuan 2013, Miller 2013, Harrison 2013). Therefore, we decided to chemically link two anti-tumor drugs, Oseltamivir Phosphate (OP) and Gemcitabine to the polymers for controlled co-delivery of chemotherapy.
Successful production of alginate based amphiphilic polymers capable of encapsulating the desired materials is a greener alternative to petroleum based polymers and could find its use in other applicable fields such as industrial controlled release systems, latexes and others.

MATERIALS AND METHODS

Alginate Preparation

High molecular weight (HMW) alginate was depolymerized in the presence of H_2O_2 and ascorbic acid according to Xiaoxia (2010).
Acidified LMW alginate was neutralized with tetrabutyl ammonium (TBA) hydroxide to give alginate-TBA salt which was confirmed by H-NMR.

'Graft From' Synthesis

Alginate-TBA was reacted with α-bromoisobutyric acid (α-BrIBA) (SET initiator) in DMF/TBAF solution via steglich esterification in presence of DCC/DMAP to give Alg-Br macroinitiator. OP was then conjugated to alginate-Br via EDAC aided coupling in water to give OP-Alg-Br. Polyethylene glycol methyl ether methacrylate (PEGMEMA), methyl methacrylate (MMA) or MMA/t-butyl acrylate was then grafted from OP-Alg-Br via SET-LRP. All steps were confirmed by H-NMR and aq-GPC.

'Graft Onto' Synthesis

$HO\text{-}PEG\text{-}NH_2$ (1.2 kDa) was reacted with $(BOC)_2O$ in water to produce HO-PEG-NH-BOC, which was esterified with α-BrIBA to give α-BrIB-O-PEG-NH-BOC initiator. MMA or MMA/t-butyl acrylate was first co-polymerized from a standard ATRP initiator (EBIB) via SET-LRP to establish appropriate reaction time and conditions. Same polymerization was then repeated with amine protected initiator to give polymer-NH-BOC which was then deprotected in acid to produce reactive amine specie (polymer-NH_2).
OP was coupled to LMW alginate as previously described to give OP-Alg-COOH, which was later reacted with polymer-NH2 via EDAC aided coupling.

RESULTS AND DISCUSSION

Reduction in MW was confirmed by dynamic light scattering (DLS) and aqueous gel permeation chromatography (aq-GPC) (Figure 1). After degradation and solubility modification LMW alginate was functionalized with SET initiator with a degree of substitution of 7%. H-NMR of macroinitiator is shown in Figure 2. Grafting from synthesis was then accomplished by SET-LRP from Alg-Br. As an example, Figure 3 shows H-NMR of the final product, Alg-pMMA co-polymer. At high concentration, the product self-assembled into micelles (average size 70nm by DLS) which could be observed by TEM. Formation of stable micelles during SET-LRP reactions provided a proof of successful synthesis of amphiphilic material capable of self-assembly in aqueous environment. Similar grafting from route is currently being followed but with OP covalently conjugated to alginate. A small amount of t-BA is now co-polymerized into the

hydrophobic polymer to provide a number of reactive sites for coupling with gemcitabine in the future.

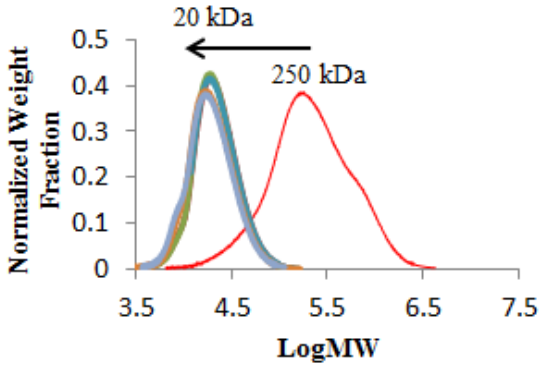

Figure 1 : aq-GPC of original and degraded alginate

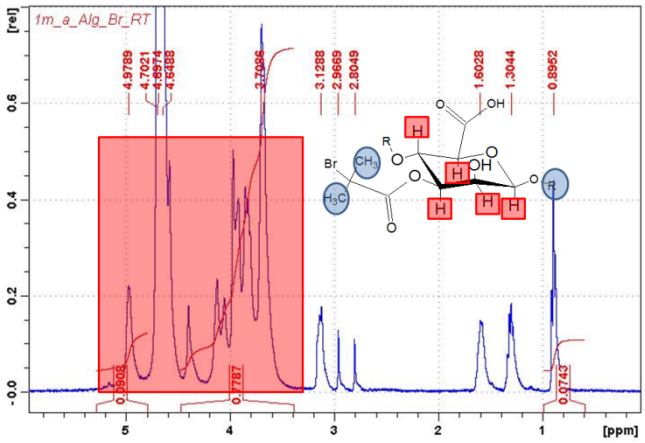

Figure 2 : H-NMR of alginate-Br macroinitiator

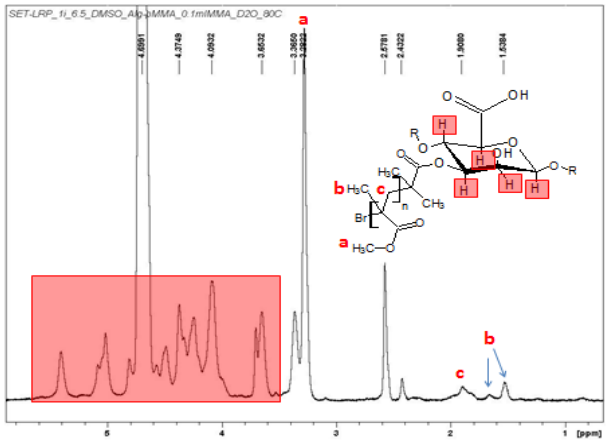

Figure 3 : H-NMR of alginate-pMMA

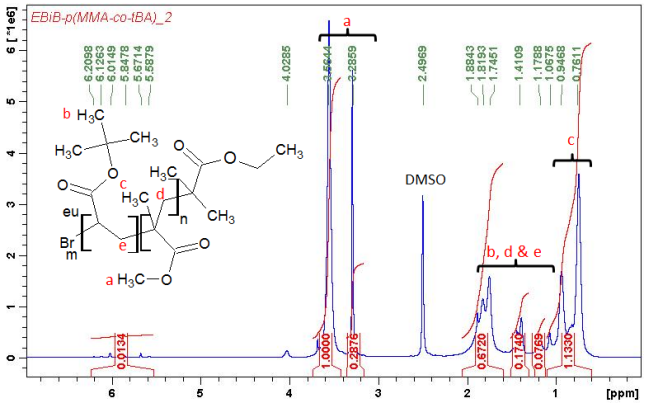

Figure 4 : H-NMR of EIB-p(MMA-co-tBA)-Br

This will result in co-delivery of the two drugs from two different regions of the micelle in a sustained fashion.

Grafting onto approach first involved preparation of hydrophobic polymer prior to linking to alginate. Figure 4 shows H-NMR of p(MMA-co-tBA) with Mw of 7-10kDa. We have finalized appropriate reaction time and conditions to give a hydrophobic fragment with a desired MW (~10kDa). In case of p(MMA-co-tBA) copolymers, as in grafting from approach, tBA is later transformed into acrylic acid which is capable of peptide bonding with Gemcitabine.
Finally, drug conjugated micelles will be tested for sustained drug release in-vitro and anti-tumor efficiency in-vivo.

CONCLUSION

Alginate based amphiphilic polymers with chemically linked drugs show a potential in the area of drug release and biomaterial engineering.

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