Di-biotin Functionalized Polymers via Atom Transfer Radical Polymerization and Click Chemistry

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Statement of Purpose: The purpose of this research is to create an injectable polymer system for tissue engineering. Our hypothesis is that by synthesizing a di-biotin-functionalized polymer, a bi-component system would result where the polymer solution could be in one syringe, a solution of avidin in another syringe, and by injecting both, a polymer network or gel would be formed. The strong affinity between biotin and naturally occurring proteins avidin and streptavidin (Kd ~ 10^{15} M) has been extensively explored[1]. These proteins have the ability to bind four molecules of biotin. As a convenient consequence, a di-biotin functionalized polymer can be used to construct a gel. Biotinylated molecules have found use in drug delivery systems and as enzyme–polymer conjugates. Di-biotin functionalized polymers have potential application as scaffolds, in drug delivery, and in biotechnology and surface engineering. Maynard et al. have previously described the in situ synthesis of streptavidin–pNIPAAm conjugates by polymerization from a protein macroinitiator[2] and a one- step procedure to synthesize low polydispersity pNIPAAm with a biotin end-group[3]. Wooley at al. described the synthesis of a biotinylated initiator for ATRP[4]. We report the synthesis of di-biotin polystyrene (PSt) and poly(ethylene oxide) (PEO) by a combination of ATRP and "click" chemistry.

Methods: *Synthesis of a biotin ATRP initiator:* The following components were combined: 1.0g biotin (4.09 mmol), 0.844g dicyclohexylcarbodiimide (DCC) (4.09 mmol), 0.05g 4-dimethylaminopyridine (DMAP) (0.409 mmol) in dimethylsulfoxide (DMSO)/dichloromethane (DCM). Finally, 0.863g hydroxyethyl bromoisobutryate (4.09 mmol) was added dropwise. The organic layer was washed three times with water. The biotin initiator was purified by column chromatography using a silica column and eluting with 1:9 methanol/chloroform (v,v). *Synthesis of mono-biotin-PSt.* Using established protocols with 10mL St (0.08727 mol), 0.083g CuBr (5.810^{-4} mol), 0.0065g $CuBr_2$ ($2.9*10^{-5}$ mol), 0.254g biotin initiator ($5.8*10^{-4}$ mol), 0.127mL pentamethyl diethylene triamine (PMDETA) ($6.11*10^{-4}$ mol), an ATRP reaction was carried out for 70 minutes at 80 °C. *Synthesis of Br-PSt-Br for click chemistry.* 50mL St (0.43 mol), 0.95mL dimethyl-2,6-dibromoheptanedionate (0.0043 mol), 0.63g CuBr (0.0043 mol), 0.049g $CuBr_2$ ($2.182*10^{-4}$ mol), and 0.96mL PMDETA (0.00458 mol), 2.5mL of toluene; 80 °C; 70 minutes yielded Mn=1900 (18% conversion); PDI=1.06. *Synthesis of N_3-PSt-N_3.* 3g Br-PSt-Br (0.00157 mol) was reacted with 0.31g sodium azide (0.00473 mol) to produce N_3-PSt-N_3. *Functionalization of biotin.* Using a similar procedure to that of the biotin initiator, 0.05g biotin, 0.0573g propargyl alcohol, 0.0589g N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride, 0.0025g DMAP was reacted in 8mL DMF. The crude product was dissolved in DCM and washed with 1M NaOH once, then washed with water four times. The product was precipitated into hexanes. *Di-biotin PSt* was prepared using the following molar ratios: biotin-acetylene (2), N_3-PSt-N_3 (1), CuBr (3) in DMF for 15 hours at r.t. In a similar manner, PEO was functionalized with azide groups, then reacted with biotin-acetylene to form *di-biotin PEO* using 0.30g N_3-PEO_{2000}-N_3 ($1.5*10^{-4}$ mol), 0.16g biotin-acetylene ($6.0*10^{-4}$ mol) 0.064g CuBr ($4.5*10^{-4}$ mol).

Results / Discussion: A biotin containing ATRP initiator was synthesized and used to produce mono-biotin PSt. NMR of the purified biotin initiator appears in Figure 1. No peaks for unreacted biotin or hydroxyethyl bromoisobutryate are seen. The synthetic route has fewer steps than that previously reported[4].

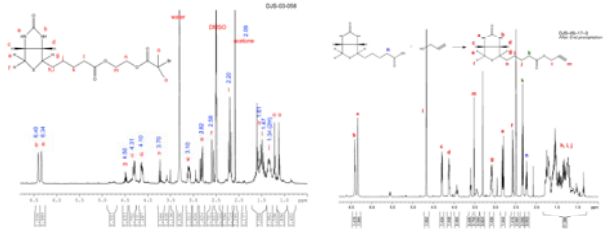

Figure 1: NMR of biotin initiator and biotin-acetylene

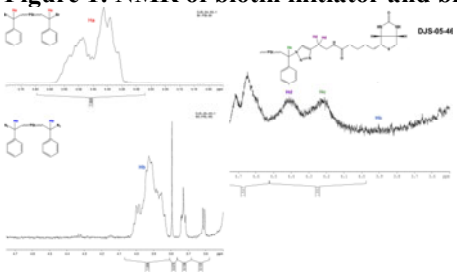

Figure 2: NMR of di-biotin PSt Synthetic Steps

Di-biotin PSt and PEO were synthesized by first making the di-bromo polymers, then converting the bromine chains ends to azido groups. Biotin was functionalized with an acetylene group. Finally, diazide PSt and PEO were reacted with biotin-acetylene to produce di-biotin PSt and PEO. The polymers were characterized by NMR (Figure 2) and GPC (not shown). Gradient polymer elution chromatography (GPEC) proved useful to characterize and separate the polymers. A gel was formed by mixing the di-biotin polymers with the protein avidin. Rheological studies of the gel were also performed.

Conclusion. This work reveals an efficient route towards di-biotin functionalized polymers by combining ATRP with click chemistry. Potential applications include use as injectable scaffolds or as strong polymer gels.

References:1) Weber PC, Ohlendorf DH, Wendoloski JJ, Salemme FR. Science 1989;243:85. 2) Bontempo D, Maynard HD. J. Am. Chem. Soc. 2005;127:6508. 3) Bontempo D, Li RC, Ly T, Brubaker CE, Maynard HD. Chem.Commun. 2005;4702. 4) Qi K, Ma Q, Remsen EE, Clark CG, Wooley KL. J. Am. Chem. Soc. 2004;126:6599.