December 17, 2008

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Polyester-*graft*-phosphorylcholine prepared by ring-opening polymerization and click chemistry†

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Received (in Cambridge, UK) 7th October 2008, Accepted 26th November 2008
First published as an Advance Article on the web 17th December 2008
DOI: 10.1039/b817600k

Phosphorylcholine (PC)-substitution on aliphatic polyesters is accomplished by click chemistry with PC-substituted azides.

Zwitterionic synthetic polymers, such as those containing phosphorylcholine (PC) moieties, exhibit excellent biocompatibility, largely due to their associated water structure and hydrophilicity that limits protein adsorption.[1] Significant efforts have been directed towards the synthesis and application of polymers from 2-methacryloyloxyethyl phosphorylcholine (MPC) for use as bio-implants and medical devices.[2–4] Aside from these methacrylate-based structures, use of the PC moiety is sparse, especially relative to hydrophilic polymers such as poly(ethylene glycol) (PEG).[5–7] Here we introduce PC groups to aliphatic polyesters, by click cycloaddition of alkyne-substituted polyesters **2** and **5** with PC-azide **3**. Through this method, properties of the PC groups are embedded within the biodegradable polyester backbone, giving materials with potential applications that benefit from a combination of biodegradability, biocompatibility, and water solubility.

Aliphatic polyesters, such as poly(ε-caprolactone) and poly(lactide), are attractive for biomedical applications due to their biodegradability and low toxicity upon degradation. However, the scope of applications for aliphatic polyesters is limited by their hydrophobicity and narrow range of facile methods for functionalization. While commercially available aliphatic polyesters lack the backbone functionality needed to readily tailor physical properties, reactive functionality can be imparted to aliphatic polyesters by polymerizing functional lactones.[8–11]

1,3-Huisgen cycloaddition "click" reactions[12,13] have proven highly suitable for post-polymerization modification of aliphatic polyesters, as the mild conditions associated with (click) chemistry allow such reactions to be carried out with little-to-no hydrolytic degradation. In addition, microwave-assisted click chemistry shortens reaction times, allowing higher conversion in some cases.[13] Polar molecules, such as azides, efficiently absorb microwave radiation; this coupled with the localized heating associated with microwave leads to higher reaction yields in shorter time-frames.[13,14]

The combination of PC-moieties with aliphatic polyesters is rare, limited to end-capped poly(ε-caprolactone) and MPC-*block*-poly(lactide) structures.[15–17] Here we synthesize PC-grafted aliphatic polyesters using click chemistry, giving water solubility to the structures by distributing the PC-grafts along the polymer backbone. Homo- and co-polymerization of α-propargyl-δ-valerolactone[11] (**1**) begins the grafting strategy illustrated in Fig. 1.

Ring-opening polymerization of alkyne **1**, with Sn(II) catalysis and benzyl alcohol initiation, gives alkyne-rich polyesters (**2**) with low polydispersity indices (PDI < 1.2) indicative of a controlled chain-growth polymerization. Alkyne-containing terpolyesters were also prepared with L-lactide and ε-caprolactone comonomers, at high temperature (170 °C) to give higher molecular weight random terpolymers (**5**). These terpolymers had PDI values of about 2, reflective of transesterification events occurring at high temperature. The

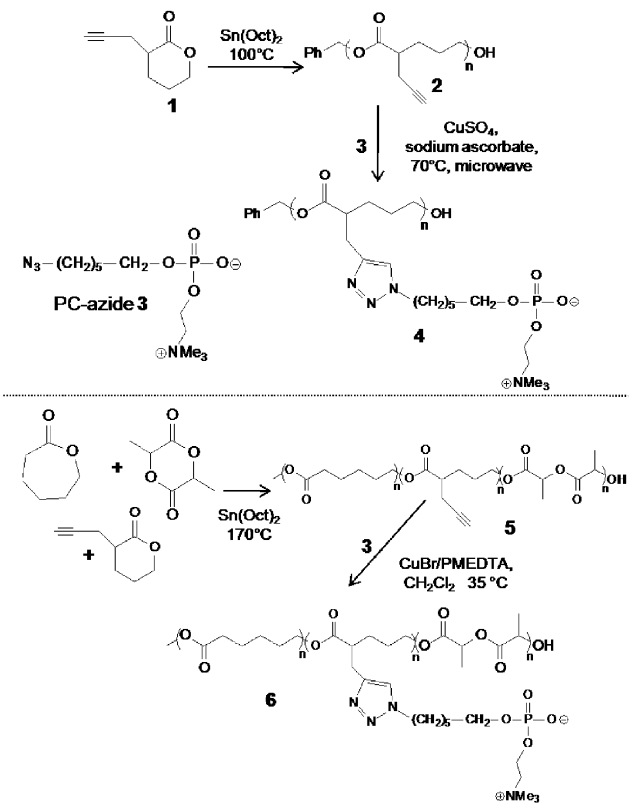

Fig. 1 Synthesis of PC-grafted polyesters: top—δ-valerolactone homopolymer **4**; bottom—L-lactide-containing terpolymer **6**.

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† Electronic supplementary information (ESI) available: Additional experimental procedures, and NMR spectra. See DOI: 10.1039/b817600k.

Table 1 Characterization of polyester-*graft*-PC

		Alkyne polyester		Polyester after PC grafting			
Entry	Polymer composition (LA–CL–AVL)	M_n[a]	PDI	%PC	M_n[b]	M_w[b]	PDI
1	0 : 0 : 100	5600	1.12	90%	4000	7000	1.37
2	0 : 0 : 100	8300	1.16	100%	10 000	13 800	1.32
3	60 : 20 : 20	48 000	2.19	20%	14 500	20 500	1.41

[a] THF GPC, polystyrene standards. [b] Aqueous GPC, PEO standards.

L-lactide–ε-caprolactone–α-propargyl-δ-valerolactone terpolymer composition was ~3 : 1 : 1, judging from integration values in the ^{1}H NMR spectrum recorded in $CDCl_3$. Both types of alkyne-containing polyesters were used for grafting PC groups by click cycloaddition of PC-azide **3**. Compound **3** was prepared by the reaction of 6-azido-hexanol with 2-chloro-1,3,2-dioxaphospholane-2-oxide (COP), followed by opening the phospholane ring of the product with trimethylamine.[18] Both microwave-assisted and traditional click chemistry were then used to graft the zwitterionic groups onto the aliphatic polyesters to give PC-polyesters **4** and **6**.

Table 1 provides molecular weight data and percent grafting for examples of alkyne-containing starting materials and polyester-*graft*-PC products. The starting polyester molecular weights ranged from 5600 to 48 000 g mol^{-1}. Entries 1 and 2 are poly(α-propargyl-δ-valerolactone) homopolymers, while entry 3 incorporates 20 mole percent of alkyne **1** with ε-caprolactone and L-lactide.

The alkyne containing polyester homopolymers were functionalized by click cycloaddition reactions performed in a water–tetrahydrofuran mixture with copper sulfate and sodium ascorbate under constant microwave irradiation at 70 °C, giving complete grafting in about five minutes. Characteristic of the completed reaction was the disappearance of alkyne protons at 2.0 ppm in the ^{1}H NMR spectrum, appearance of triazole signals at 7.7 ppm, and methyl group signals of the PC-moiety at 3.2 ppm. The purified polyester-*graft*-PC structures were typically obtained in yields of 60% or greater as off-white powders. Upon storage for weeks, the polyester-*graft*-PC polymers absorb water and appear waxy. Lyophilization recovers the powder.

The L-lactide containing terpolymers required different click conditions, due to lower solubility of the starting material and the greater hydrolytic lability of the lactide-rich structures. For the terpolymers, the click reactions were performed in dichloromethane with CuBr–PMDETA. The initially homogenous reaction mixture became cloudy over the course of the reaction, as the solubility of the polymer in dichloromethane decreased with increasing PC-substitution. Nonetheless, the PC moiety was found to be incorporated completely into the structure, with isolated polymer yields of nearly 70%. Similar to the homopolymers, the terpolymers were isolated as off-white powders.

The polyester-*graft*-PC products were purified by treatment with Cuprisorb™ to remove copper, and dialysis in water. While the alkyne polyester starting materials **2** and **5** are hydrophobic, and PC azide **3** is amphiphilic, the polyester-*graft*-PC materials are found to give homogeneous solutions in water only, and are not soluble in most organic solvents. The GPC traces of these polyesters were monomodal, Gaussian curves. For the low molecular weight examples, the relative molecular weights obtained by GPC were in quite reasonable agreement with the expected molecular weights. The higher molecular weight terpolymer with lower grafting density gave a lower than expected molecular weight by GPC, likely due to the effect of a collapsed structure in water.

Fig. 2 shows the gel permeation chromatogram (refractive index detection) of the polyester-*graft*-PC with a number average molecular weight (M_n) of 10 000 g mol^{-1} and PDI of 1.3 based on PEO standards (entry 2 in Table 1; methanol flow marker at ~34 minutes). The relatively low polydispersity index reflects the clean click chemistry possible in these systems. While there are changes in the PDI in the polyester-*graft*-PC product relative to their respective alkyne-containing starting materials, this is not likely a result of significant degradation; confirmed by the absence of ^{1}H NMR spectrum signals at 4.5 ppm that typify degradation.

Fig. 3 shows the ^{1}H and ^{31}P NMR spectra of a representative polyester-*graft*-PC sample. In the ^{1}H NMR spectrum, the click cycloaddition is confirmed by the appearance of the triazole signal at 7.7 ppm, the PC *N*-methyls at 3.2 ppm, and the absence of an alkyne proton signal (otherwise at 2.0 ppm). The ^{31}P NMR spectrum (Fig. 3b) shows a single resonance

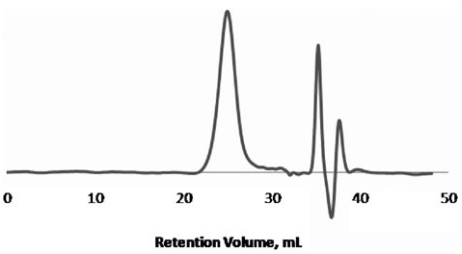

Fig. 2 Aqueous GPC trace of PC-grafted polyester (Table 1, entry 2).

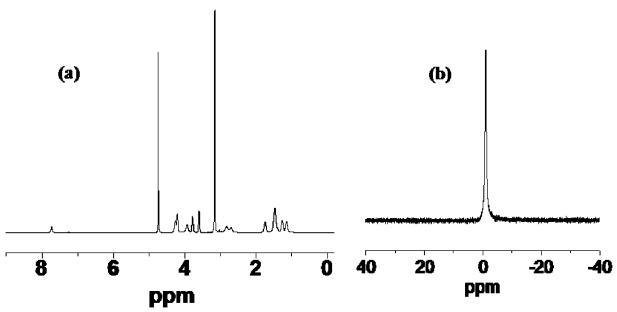

Fig. 3 (a) ^{1}H and (b) ^{31}P NMR spectra of polyester-*graft*-PC (Table 1, entry 2).

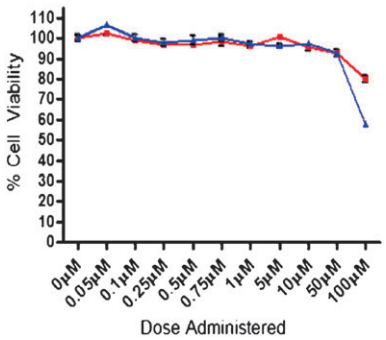

Fig. 4 CellTiter-Glow luminescent cell viability assays of polyester-*graft*-PC (Table 1, entry 2) after 24 (red) and 48 hour (blue) incubation in MCF7 cell culture.

corresponding to the attached PC group at −0.4 ppm. This resonance is shifted slightly upfield from that of PC-azide **3**.

Finally, the PC-grafted aliphatic polyesters were evaluated for cytotoxicity in cell culture. For example, as shown in Fig. 4, the cell viability assay of the human breast cancer cell line MCF7 (American Type Cell Culture) performed in the presence of PC-grafted polyester showed good cell viability, determined using the CellTiter-Glow luminescent assay (Promega), as expected for these PC-containing macromolecules. With polymer concentrations under 100 μM, very little cell death was found at 24 or 48 hours; cell viability fell off at higher concentrations. The ability to safely use these PC-polyesters at appropriate concentrations will open potential routes for their *in vivo* applications.[19–21]

In summary, we have presented the synthesis and characterization of water-soluble, biodegradable, zwitterionic aliphatic polyesters using ring-opening polymerization and post-polymerization click chemistry with PC-azide **3**. The phosphorylcholine moiety imparts hydrophilicity to the polyester, and can be viewed as a PEG alternative. The biocompatibility of PC-functionalized aliphatic polyesters suggests their usefulness for integration into medical devices, biomaterials, and drug delivery vehicles.

The authors thank Darren Hamilton and Kenneth Carter for providing access to microwave heating, Kimberly Wojeck for assistance with cell culture and the National Science Foundation (MRSEC and CBET-0553957), Abbott Vascular, and the Pioneer Valley Life Science Initiative for funding.

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