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Hybrid Dendritic−Linear Polyester−Ethers for in Situ Photopolymerization

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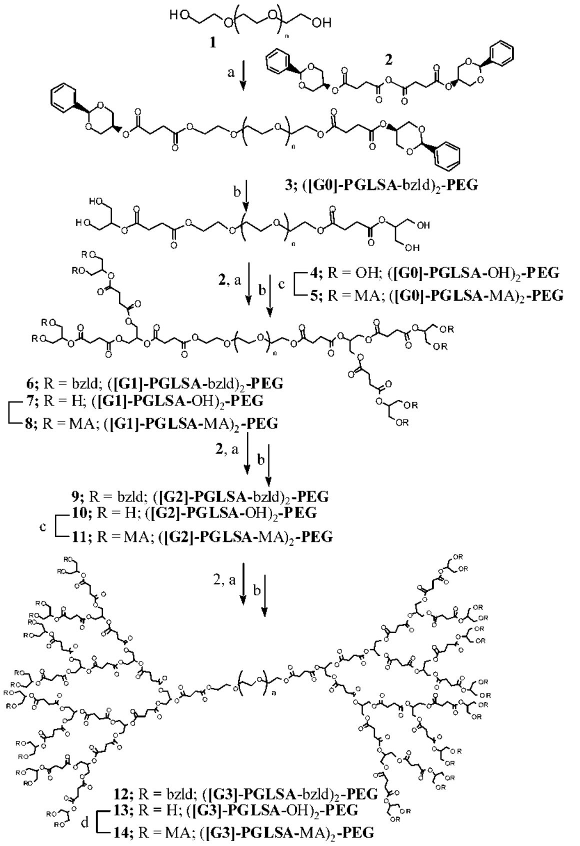
Received January 12, 2002

Dendritic macromolecules possess unique chemical and physical properties, and are of interest for a wide-range of industrial and pharmaceutical applications.[1−17] These monodisperse macromolecules possess a highly branched repeating structure, a high surface area-to-volume ratio, numerous terminal ends and adopt globular structures in solution.[2−7,10,12] Hybrid dendritic−linear copolymers are less explored, but offer additional opportunities for material property optimization through discrete changes at the molecular level. We are designing, synthesizing, and investigating novel dendritic macromolecules composed entirely of building blocks known to be biocompatible or degradable in vivo to natural metabolites. Such polymers may provide unique solutions to challenges in the medical and tissue engineering fields,[18−25] where spatial orientation and/or high ligand density are desired on the macromolecular scale. Herein, we report the divergent synthesis and characterization of novel first through fourth generation (G0−G4) hybrid dendritic−linear polyester−ethers composed of poly(ethylene glycol), glycerol, and succinic acid, and the use of these polymers to seal corneal lacerations.

Schemes 1 and 2 show the synthesis of new poly(glycerol-succinic acid)-poly(ethylene glycol) hybrid dendritic−linear copolymers **([Gn]-PGLSA)$_2$-PEG**. This divergent synthetic strategy[15,16,26,27] builds upon our recent success in preparing polyether−ester and polyester dendrimers[28,29] using the benzylidene acetal (bzld) protecting group, although using a different esterification method. The molecular weight ratio of the linear to dendritic block in these macromolecules is small as to maintain dendritic character in the macromolecule while imparting greater aqueous solubility. This is a departure from the majority of previous hybrid linear−dendritic copolymers reported, which possess a large linear block compared to the dendritic block.

Scheme 1. Divergent Synthesis of G1, G2, and G3 Dendrimers Composed of Poly(ethylene glycol), Glycerol, and Succinic Acid[a]



[a] Reagents and Conditions (a) DMAP, CH_2Cl_2, 25 °C, 14 h. (b) H_2, $Pd(OH)_2/C$, CH_2Cl_2/CH_3OH (2/1) 25 °C, 8 h. (c) methacrylic anhydride, CH_2Cl_2, DMAP, 25 °C, 14 h; (d) methacrylic anhydride, THF, DMAP, 25 °C, 14 h.

Several esterification strategies were explored to optimize the reaction yields and simplify purification, including DCC/DPTS, NHS-activated ester in the presence of TEA, and anhydride coupling in the presence of DMAP. The first two approaches met with limited success. The G0 and G1 dendrimers were prepared, but further reactions yielded incomplete dendrimer generations as determined by NMR and MALDI-MS. Using an anhydride coupling approach, as described by Fréchet,[30] we successfully synthesized a series of **([Gn]-PGLSA)$_2$-PEG** hybrid dendritic−linear copolymers. The activated monomer was prepared in two steps by first treating *cis*-1,3-*O*-benzylidene glycerol with succinic anhydride in pyridine to obtain succinic acid mono(2-phenyl-[1,3]dioxan-5-yl) ester. The monoester was then treated with 0.6 equiv of DCC in dichloromethane (DCM) to afford the anhydride of succinic acid mono-(2-phenyl-[1,3]dioxan-5-yl) ester, **2**, after precipitation in hexanes (95% yield). The tetrafunctional core, **([G0]-PGLSA**-OH)$_2$**-PEG**, **4**, was synthesized in two steps by treating PEG (3400 M_w) with 6 equiv of the anhydride and 0.6 equiv of DMAP in DCM. The reaction was quenched with *n*-butanol and washed with saturated sodium bicarbonate, and then the product, **([G0]-PGLSA**-bzld)$_2$**-PEG**, **3**, was precipitated from ethyl ether (91% yield). The protected core and 20% palladium hydroxide on carbon, $Pd(OH)_2/C$, were added to a 2:1 DCM/methanol solution and shaken at 60 psi of hydrogen to obtain **4** in 97% yield. **([G1]-PGLSA**-OH)$_2$**-PEG**, **7**, was synthesized using similar conditions except 4 equiv of the anhydride were used for each alcohol (93% and 93% yields, respectively). Reiteration of the esterification (4 equiv of **2** per alcohol; 91% yield) and deprotection (98% yield) reactions afforded **([G2]-PGLSA**-bzld)$_2$**-PEG**, **9**, and **([G2]-PGLSA**-OH)$_2$**-PEG**, **10**, respectively. The **([G2]-PGLSA**-bzld)$_2$**-PEG** was precipitated from ethyl ether. The third generation block polymer, **([G3]-PGLSA**-OH)$_2$**-PEG**, **13**, was prepared following another round of esterifi-

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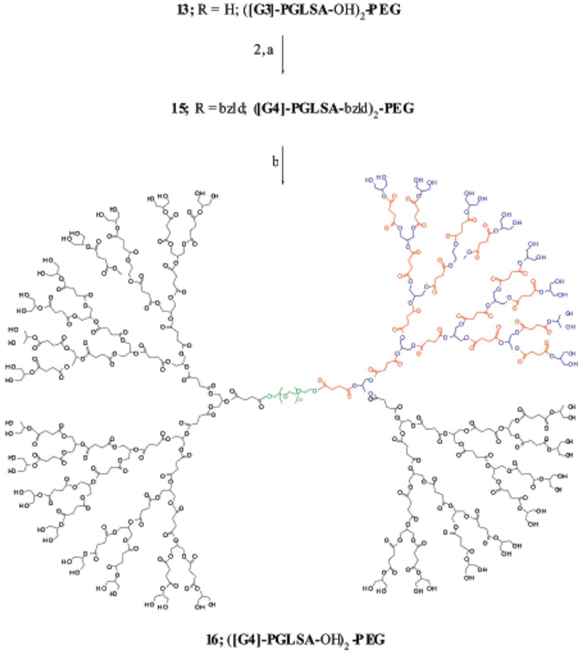
10.1021/ja025576y CCC: $22.00 © 2002 American Chemical Society

Scheme 2. Divergent Synthesis of the G4 Dendrimers Composed of Poly(ethylene glycol), Glycerol, and and Succinic Acid (Green = PEG; Red = Succinic Acid; Blue = Glycerol)[3]



[a] Reagents and Conditions: (a) DMAP, THF, 25 °C, 14 h. (b) H_2, $Pd(OH)_2Cl_2/CH_3OH$ (2/1), 25 °C, 8 h.

cation and deprotection reactions in similarly high yields (92% and 98%, respectively). Again, **([G3]-PGLSA**-bzld**)$_2$-PEG**, **12**, was isolated by precipitation in ethyl ether. Finally **([G4]-PGLSA-**bzld**)$_2$-PEG**, **15**, was prepared by treating **13** with 128 equiv of **2** in the presence of DMAP. After quenching the reaction and washing with saturated sodium bicarbonate, the hybrid dendritic−linear polymer was isolated in ethyl ether (87% yield). The deprotected macromolecule, **([G4]-PGLSA**-OH**)$_2$-PEG**, **16**, was isolated after hydrogenolysis (98% yield). The photocross-linkable derivatives of the hybrid dendritic−linear copolymers **5**, **8**, **11**, and **14** were prepared by reacting the hydroxyl functionalized copolymers with methacrylic anhydride in the presence of DMAP (yields 89−93%).

The PEG block of this aliphatic ABA triblock copolymer serves as a diagnostic NMR tag to monitor the change in proton integrations for each successive generation. The FTIR spectrum of each deprotected copolymer contains the expected broad O−H stretch at ~3400 cm^{-1}, not present before hydrogenolysis. Molecular weight data determined by MALDI MS and SEC for the benzylidene protected and deprotected G0 through G4 **PGLSA-PEG** copolymers are summarized in Table 1. The protected and deprotected G0 through G2 **PGLSA-PEG** copolymers are soluble in common aqueous and organic solvents. The protected G3 and G4 **PGLSA-PEG** copolymers are soluble in halogenated solvents, THF, and ethyl acetate. The deprotected G3 and G4 **PGLSA-PEG** copolymers are soluble in polar solvents such as in DMF, EtOH, and H_2O.

Photocross-linkable hybrid dendritic−linear copolyester−ethers are of interest for tissue engineering applications where in situ photopolymerization of a polymer to afford a temporary cell scaffold can aid tissue growth or repair.[31] This new surgical modality is being explored with linear polymers in cardiology,[32] ophthalmology,[33,34] and orthopaedics.[35,36] We envision that in situ photopolymerization will be particularly useful when the damaged tissue site is not easily accessible or removal of sutures at a postoperative date is unfavorable. Full-thickness corneal lacerations are one clinical indication that is likely to benefit from a sutureless wound closure procedure since the use of current monofilament nylon sutures inflicts additional trauma to the wound and can be associated with a number of post-operative complications including microbial keratitis, extended healing time, and astigmatism.[37−39]

Table 1. MALDI-MS and SEC Data for Gn-PGLSA-PEG Copolymers

no.	calcd M_w	MALD^a M_n	SEC^b M_n	PDI
1	3366	3351	3550	1.03
3	3891	3875	3750	1.04
4	3714	3696	3500	1.04
5	3957	3914	3520	1.04
6	4763	4749	3790	1.04
7	4411	4394	4440	1.03
8	4956	4897	3740	1.04
9	6509	6492	4680	1.04
10	5804	5788	5210	1.03
11	6893	6792	4390	1.04
12	10000	9985	6900	1.02
13	8591	8575	7820	1.03
14	10773	10498	6820	1.03
15	16983	16968	7950	1.02
16	14163	14146	8980	1.02

[a] All PDIs are 1.02. [b] Relative molecular weights by size exclusion chromatography with PEG standards.

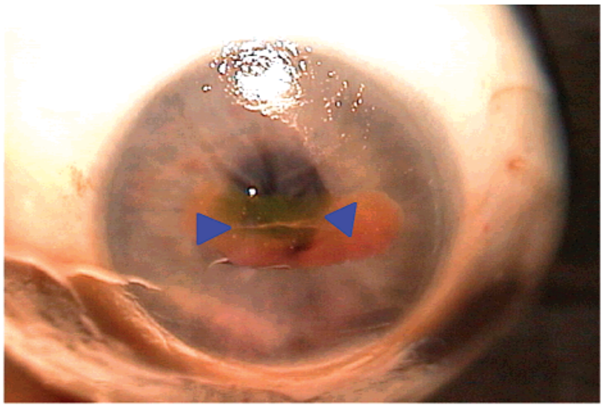


Figure 1. Optical image of an eye with a sealed 4.1 mm linear laceration using copolymer **8**. The sealed laceration is the horizontal line between the two arrowheads.

An enucleated eye with the cornea facing upward was held under a surgical microscope and a 4.1 mm laceration was made with a keratome blade. This wound was then closed using either three interrupted 10−0 nylon sutures in a standard 3-1-1 suturing configuration or the photocross-linkable biodendritic copolymer (see Scheme 1). Specifically, 4 μL of copolymer **5**, **8**, **11**, or **14** was applied to the laceration and argon ion laser irradiation produced the dendritic gel sealing the wound (Figure 1; 200 mW, 1 s exposures; 50 s total irradiation time; the polymer solution contained ethyl eosin in 1-vinyl pyrrolidinone and TEA as photoinitiator and cocatalyst). Next, saline was injected in the anterior chamber via a syringe inserted through the scleral wall adjacent to an ocular muscle until the repaired laceration leaked. A cardiac transducer probe inserted approximately 1 cm through the optic nerve monitored the leaking pressure for both the nylon suture ($N = 6$) and biodendrimer sealant ($N = 3$; for each copolymer tested) treated eyes. For reference, normal intraocular pressure in a human eye is between 15 and 20 mmHg. In this in vitro study, the mean leaking pressure (LP) for the suture treated eyes was 90 ± 18 mmHg. The LP for the eyes sealed with copolymer **8** was 171 ± 44 mmHg (range 142−222 mmHg). Copolymer **5** did not gel adequately to seal the wound and leaked before measurements could be obtained. Copolymer **11** polymerized too quickly under the operating microscope to be delivered to the wound in a controlled fashion

(LP < 15 mmHg). Copolymer **14** was insoluble in water and only slightly soluble in alcohols. Copolymer **14** precipitated slightly when applied to the laceration and did not seal the wound.

The hybrid linear−dendritic copolymer, **8**, seals the wound better than conventional sutures and can withstand greater pressures and stresses placed on or around the wound site. The procedure with **8** is approximately five times faster than suturing the wound, potentially reducing surgical time and intervention. Moreover, the cross-linked gel of **8** is transparent, elastic, and adhesive—favorable properties for an ophthalmic sealant. The tissue sealing mechanism with **8** is likely one of physical entrapment where an interpenetrating network (IPN) is formed between the cross-linked copolymer and the tissue. This is substantiated by the observation that the copolymer does not seal the wound without laser polymerization. If the copolymer is too hydrophobic an IPN does not form, as with copolymer **14**. With these concepts in mind, we are currently designing other dendritic structures that possess high end-group functionality while balancing the hydrophilic/hydrophobic properties of the macromolecule to favor interpenetrating network formation for subsequent tissue sealing.

In summary, novel aliphatic polyester−ether hybrid dendritic−linear polymers composed of poly(ethylene glycol), glycerol, and succinic acid are synthesized using an efficient and high yield divergent procedure. Once prepared, these dendritic macromolecules can be further functionalized to contain photocross-linkable groups. The photocross-linkable dendritic gels possess sufficient tissue adhesive properties to seal corneal lacerations. These polyester−ether ABA−triblock copolymers further expand the polymers available for study. Tailoring the linear and dendritic blocks will afford macromolecules with unique and interesting chemical, physical, and mechanical properties. Such polymers are likely to facilitate the design, development, and use of new biomaterials for specific tissue engineering applications.

Acknowledgment. This work was supported by the Pew Scholars Program in the Biomedical Sciences, the Johnson and Johnson Focused Giving Program, and the NIH (ROI-EY 13881). We thank the North Carolina Eye Bank. M.A.C. gratefully acknowledges the NIH Biological Chemistry Training Grant Program at Duke University. M.W.G. also thanks the Dreyfus Foundation for a Camille Dreyfus Teacher-Scholar, the 3M corporation for a Non-Tenured Faculty Award, and the Alfred P. Sloan Foundation for a Research Fellowship.

Supporting Information Available: Detailed experimental information and characterization data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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JA025576Y