Pure Appl. Chem., Vol. 76, Nos. 7–8, pp. 1375–1385, 2004.

Dendritic polymers composed of glycerol and succinic acid: Synthetic methodologies and medical applications*

Nathanael R. Luman[1], Terry Kim[2], and Mark W. Grinstaff[1,‡]

[1]*Departments of Chemistry and Biomedical Engineering, Metcalf Center for Science and Engineering, Boston University, Boston, MA 02215, USA;* [2]*Department of Ophthalmology, Duke University Medical Center, Durham, NC 27710, USA*

Abstract: Research on dendrimers continues to expand as further advances in synthetic methodology and characterization techniques translate to additional applications. Recently, dendritic polymers composed of the natural metabolites glycerol and succinic acid were synthesized, functionalized, and evaluated as new medical materials. The design and synthesis of poly(glycerol-succinic acid) dendritic polymers are discussed, including comparing and contrasting the convergent vs. the divergent methodologies for preparing such macromolecules. Finally, a photocrosslinkable dendritic macromolecule was prepared and successfully used to close linear corneal lacerations as well as to secure LASIK flaps.

Over the past century, the field of polymer chemistry has expanded and evolved to include numerous classes of polymers. Today, polymers can be synthesized with specific chemical, physical, and medicinal properties that suit a diverse set of applications ranging from medical devices to familiar consumer products. Dendrimers are monodisperse, hyper-branched polymers possessing three main structural zones consisting of a central core, internal branching layers, and peripheral end groups. The monomer units are assembled in a controlled step-growth polymerization process and are organized in layered "generations". The branched structure of dendrimers affords a globular, three-dimensional macromolecular shape and generally imparts a set of properties including high solubility, low viscosity, adhesivity, and glass-transition temperatures which differ from the corresponding linear analog [1–9]. As a consequence of the macromolecular structure and resulting properties, dendrimers are being evaluated in systems ranging from chemical catalysis to clinical therapies [10–18].

Dendritic polymers are being explored in a variety of medical applications owing to their size, ease of functionalization, large number of surface end groups, and well-defined structure [19]. Specifically, dendrimers are being investigated as magnetic resonance imaging (MRI) contrast agent carriers, gene transfection vehicles, drug delivery systems, and tissue sealants [20–23]. For example, a dendrimer possessing approximately 170 Gd^{3+} ions gave enhanced in vivo MR images of organs and blood vessels as well as longer circulation times compared to low-molecular-weight chelators. This poly(amidoamine) (PAMAM) dendrimer was terminated with 2-(4-isothiocyanatobenzyl)-6-methyldiethylenetriaminepentaacetic acid (DPTA) chelating groups for gadolinium ion coordination [24]. Dendritic poly(lysine) and other polycationic dendrimers form electrostatic complexes with DNA

*Lecture presented at the symposium "Controlling the self assembly in macromolecular systems: From nature to chemistry to functional properties", as part of the 39th IUPAC Congress and 86th Conference of the Canadian Society for Chemistry: Chemistry at the Interfaces, Ottawa, Canada, 10–15 August 2003. Other Congress presentations are published in this issue, pp. 1295–1603.

‡Corresponding author

and are of use for gene delivery [25,26]. The efficiency of dendrimer-DNA complexes transferring genes into cells is actively being examined, and a commercial product is currently sold [27]. As drug delivery systems, dendrimers provide an internal space for encapsulation of therapeutic agents or serve as a pro-drug through chemical attachment. The dendritic interior regions contain "pockets" that are capable of localizing hydrophobic molecules. This phenomenon has been demonstrated by a number of dendritic systems including poly(glycerol-succinic acid) PGLSA dendrimers [28–31]. The end groups of dendrimers have been covalently functionalized with multiple copies of drug molecules such as methotrexate (MTX) [32,33]. Dendrimers are also being investigated for use as tissue sealants where conventional suturing or other surgical closure methods are not ideal [34]. Recently, full-thickness corneal lacerations in enucleated eyes have been successfully repaired using photocrosslinkable dendritic macromolecules [35].

The role of dendrimers in the medical field began at the basic research level, has advanced to such specific material-based applications mentioned above, and has now reached the stage of U.S. Food and Drug Administration (FDA) approved clinical trials. Dendritic Nanotechnologies and Starpharma have been granted authorization for Phase I clinical trials of Vivagel^{TM}, a dendrimer-based anti-HIV/AIDS product [36]. Given the clinical progress and the continued strong research effort in dendrimers, including systems specifically designed with biocompatibility in mind, it is likely that these macromolecules will enhance existing therapeutic practices or provide new lines of patient treatment.

Recently, the Grinstaff laboratory has reported the synthesis and characterization of polyester dendrimers and dendrons composed entirely of glycerol and succinic acid. These dendritic polymers are termed "biodendrimers" since the building blocks are known to be biocompatible or degradable in vivo to natural metabolites. Biodendrimers have been prepared using either a divergent [37,38] or convergent [39] synthetic methodology. Both methodologies can afford the same final macromolecular structure, or the synthetic routes can be utilized separately to obtain unique, functionalized dendritic polymers. PGLSA dendrimers, dendrons, and their derivatives are well suited for bioengineering and medical applications as a consequence of the composition and resulting physical and chemical properties. To this end, we are investigating these polymers as carriers for anticancer drugs [31], cell scaffolds [40], and tissue sealants [35,41]. This manuscript will address monomer selection, ester linkages, and the specific advantages of each synthetic methodology for the preparation of biodendritic macromolecules. Moreover, we will highlight our recent successful in vitro and in vivo results using a photocrosslinkable derivative to repair corneal lacerations and secure LASIK flaps.

Glycerol and succinic acid, when joined together as shown in Fig. 1, represent the repeat unit present in PGLSA biodendrimers. Both of these natural compounds are abundant and inexpensive starting materials. Glycerol (**1**), also known as 1,2,3-propanetriol or glycerin, is the simplest trihydric alcohol and is a clear, viscous, hydroscopic liquid at room temperature (m.p. = 17.8 °C, b.p. = 290.0 °C). It is miscible with water and alcohol while slightly soluble in ether or ethyl acetate. Glycerol is generally regarded as safe (GRAS) by the FDA. Glycerol is used as a general purpose food additive as well as an ingredient in many medicinal and commercial products (e.g., ointments, creams, lotions, toothpastes, and jellies) [42].

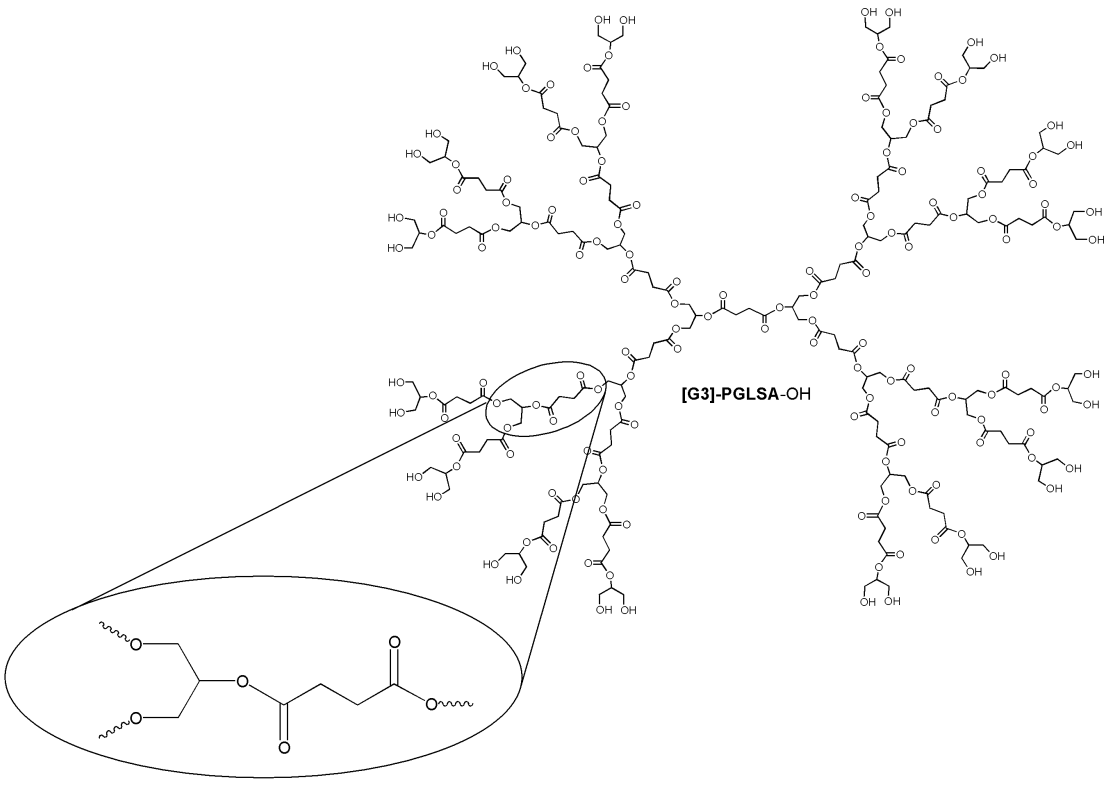


Fig. 1 Repeat unit of PGLSA biodendrimers.

Glycerol occurs naturally in all plant and animal cells as a component of triacylglycerides or phospholipids (Fig. 2). Triacylglycerides are especially abundant in adipose tissue and are used by cells for energy storage and heat insulation. Triacylglycerides are under hormonally controlled catabolism according to the energy requirements of the cell and can be broken down to glycerol and fatty acid units; the latter is involved in cellular production of adenosine triphosphate (ATP). Additionally, glycerol can be converted to glucose through a series of chemical transformations in the liver [43].

Succinic acid (**2**; butanedioic acid) is a four-carbon dicarboxylic acid that is solid at room temperature (m.p. = 187 °C, b.p. = 235 °C). It is soluble in water, alcohol, acetone, and ether. Like glycerol, succinic acid is on the GRAS list and it is used as a flavor enhancer, pH control agent in food products, and as an ingredient in toothpastes [44]. Succinic acid is found in vivo as its dianionic form, succinate, in the mitochondrial matrix of cells that undergo aerobic respiration [43].

In addition to being biological metabolites, glycerol and succinic acid possess chemical functionality and reactivity differences. The branched structure of glycerol naturally lends itself toward the preparation of a dendritic AB_2 monomer. The reactivity difference between the C1 and C3 primary hydroxyls vs. the central, secondary C2 hydroxyl can be exploited to selectively modify the molecule. For our purposes, the two primary alcohols of glycerol were protected with benzaldehyde to form the acetal, *cis*-1,3-*O*-benzylidene glycerol (**3**) so that the remaining hydroxyl group can be independently functionalized. This reaction is catalyzed by sulfuric acid and the *cis* isomer of the six-membered ring is isolated preferentially over other by-products [45]. The benzylidene acetal serves as a protecting group that can be removed under mild conditions with hydrogen and a palladium catalyst [46,47]. Since the more reactive primary alcohols are protected by the benzylidene acetal, the less reactive secondary alcohol can be esterified by a ring opening esterification with succinic anhydride in pyridine. The prod-

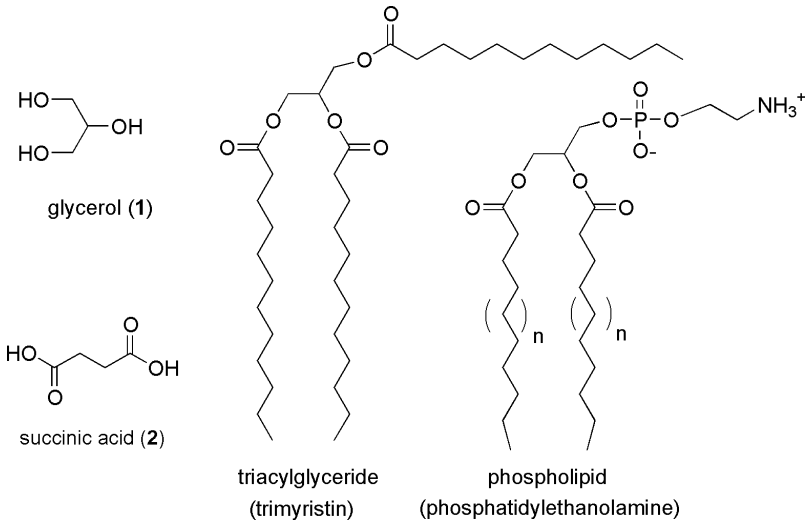


Fig. 2 Chemical structures of glycerol, succinic acid, trimyristin, and phosphatidylethanolamine.

uct of this reaction, 2-(*cis*-1,3-*O*-benzylidene glycerol) succinic acid monoester (**9**), is the monomer used in the synthesis of PGLSA biodendrimers.

These PGLSA biodendrimers are polyesters. Since ester bonds are hydrolytically unstable, these materials will degrade over time under physiological conditions. Controlling the lifetime of an implanted material is of particular importance in designing a range of biomedical products. Ester hydrolysis proceeds slowly at neutral conditions and is catalyzed enzymatically and by acid or base. Consequently, polyester materials are used as absorbable temporary scaffolds for tissue engineering and drug delivery [48–50]. If designed properly, these polymeric materials will degrade through hydrolytic scission to monomer units that will be metabolized or excreted on the timescale appropriate for a beneficial medical outcome.

For example, linear poly(lactic acid) (PLA) (Fig. 3) is a polyester composed of the natural monomer, lactic acid. PLA is an FDA-approved polymer used in the clinic as well as for basic bio-

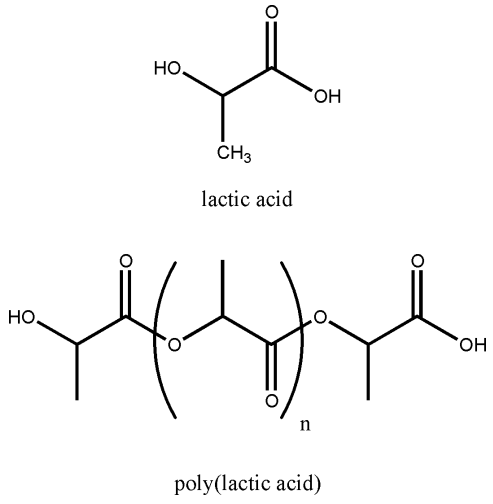


Fig. 3 Lactic acid and poly(lactic acid).

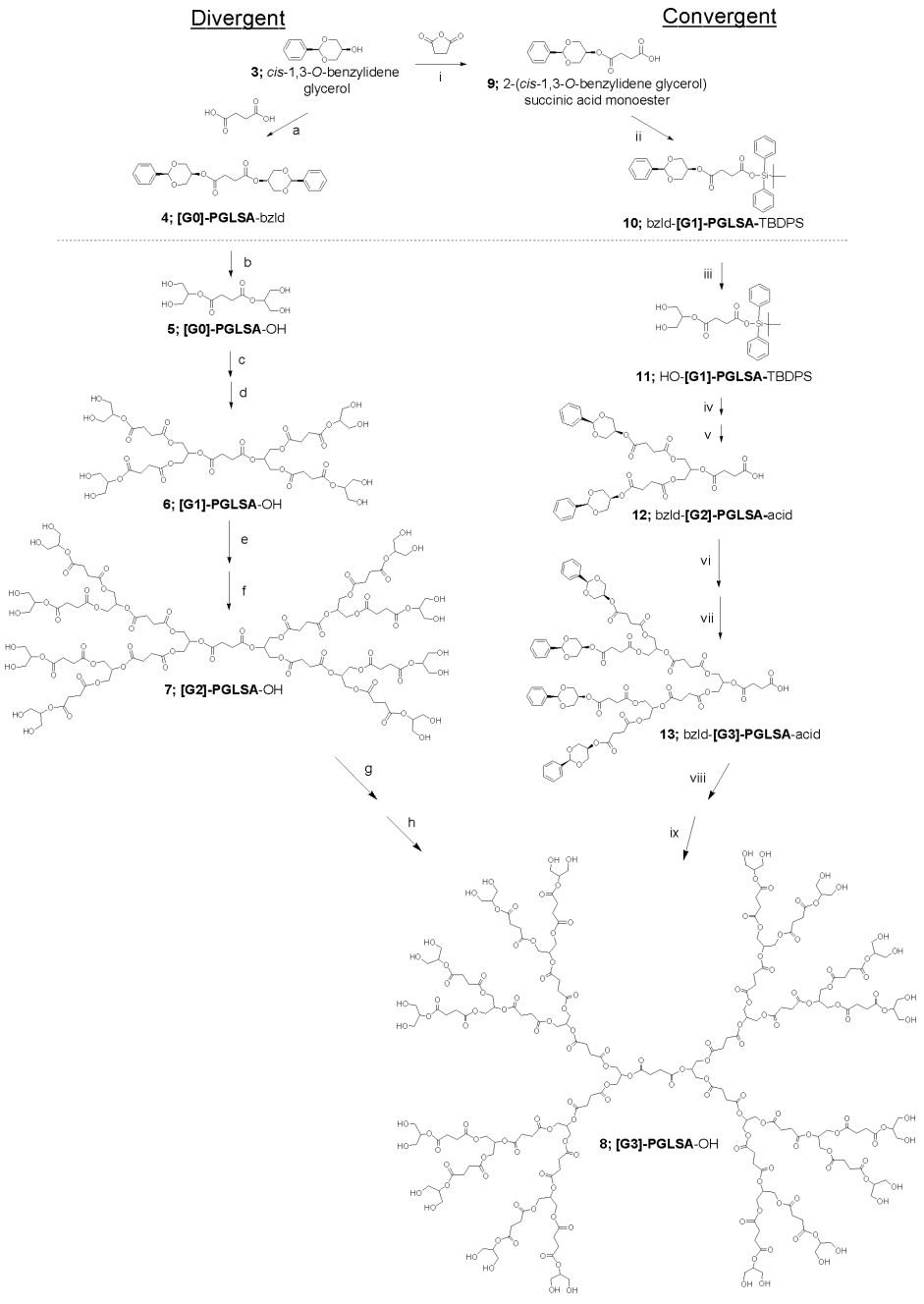
medical research to form sutures, bone fixation plates and screws, and controlled release drug delivery systems [51–53]. Lactic acid has been copolymerized with monomers such as lysine to produce functionalized polymers for use as tissue engineering scaffolds [54,55]. The rate of polyester degradation is dependent upon many factors including molecular weight, polydispersity, morphology, size, porosity, polarity, surface area, and degree of crystallinity. Yet, control over these properties and the ability to functionalize linear PLA are limited. Dendritic polyesters offer additional means of manipulating many of these properties with degrees of precision unusual for conventional linear polymers.

The divergent and convergent synthesis of PGLSA dendrimers and dendrons are shown in Scheme 1. The left-hand side of this scheme depicts the divergent methodology and is generally representative of the synthetic route used by our laboratory to prepare biodendrimers. The divergent synthesis begins by coupling two equivalents of *cis*-1,3-*O*-benzylidene glycerol (**3**) to succinic acid using dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)-pyridinium *p*-toluenesulfonate (DPTS) to produce a protected core, **4**. DCC is a dehydrating reagent, and its effectiveness is enhanced by the weakly acidic catalyst, DPTS. The ureal DCU adduct formed as a by-product from this reaction precipitates from the reaction mixture. This precipitation process simplifies the purification of the dendrimers since the by-products are removed by filtration. Compound **4** can be converted into a tetrahydroxy core through simultaneous hydrogenolysis of both acetals under a hydrogen atmosphere in the presence of either Pd/C or $Pd(OH)_2/C$. These two steps, DCC coupling followed by hydrogenolysis, occur in high yields and are subsequently repeated to construct larger dendrimers. Details of the experimental reaction conditions can be found in our recent publications [37,38].

The divergent synthesis is convenient for large-scale dendrimer synthesis, and reactions can be performed on the decagram scale. Each successive esterification procedure requires an exponential increase in the number of reactions on the dendritic periphery, a consequence that quickly adds mass to the growing dendrimer. The large difference in molecular weight between the dendrimer and reactants allows for easy purification of the desired product, usually by precipitation in cold ether. The esterification and hydrogenolysis reactions of each generation are monitored by ^{1}H NMR spectroscopy. The coupling reactions change the relative integrated areas of the aromatic, glycerol, and succinic acid peaks, and for the deprotection reactions a disappearance of aromatic protons is observed. Our laboratory has synthesized up to a fifth-generation PGLSA dendrimer by this technique, and the products have been characterized by ^{1}H NMR, ^{13}C NMR, size exclusion chromatography (SEC), mass spectrometry (MS), and elemental analysis (EA).

In addition to DCC/DPTS esterification, we have found that the symmetrical anhydride, **14** (Fig. 4), is a useful reagent for dendrimer synthesis when used in conjunction with dimethylaminopyridine (DMAP) [35,56]. This anhydride coupling reaction allows for convenient removal of by-products via acidic and basic aqueous washes. Modifications to divergently synthesized dendrimers are possible at two main positions. Either the periphery can be derivatized postsynthetically or different moieties may be initially incorporated at the core. PGLSA biodendrimers terminated with alcohols, carboxylic acids, and crosslinking groups such as methacrylate and monomethallyl esters have been synthesized. A series of dendritic-linear hybrid (ABA tri-block) macromolecules with various molecular weights of poly(ethylene glycol) incorporated into the PGLSA core have also been prepared using this divergent strategy [35].

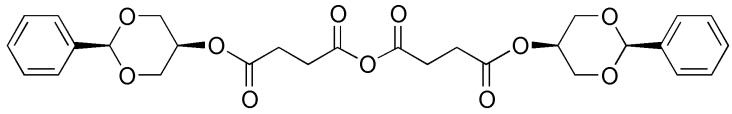
The right side of Scheme 1 shows the convergent synthesis of a third-generation PGLSA dendrimer. This synthetic approach enables more intricate engineering and control of the dendritic structure. For example, the convergent synthesis allows precise single point variations at either the surface or interior layers as well as the preparation of block and surface-block dendrimers [14,57]. The convergent approach gradually links surface units together to form dendrons. Eventually, these dendrons are attached to a multifunctional core to afford a dendrimer. In order to synthesize PGLSA dendrimers convergently, two orthogonal protecting groups are required: one at the periphery and another at the focal point. The carboxyl group of succinic acid was selected as the focal point in order to maintain similar coupling and deprotection reactions (DCC/DPTS esterification; Pd/C catalyzed hydrogenolysis) as



Scheme 1 Divergent and convergent synthesis of **[G3]-PGLSA-**OH dendrimer: (a) DCC, DPTS, DCM, RT, 14 h, 90 % yield; (b) 50 atm H_2, Pd/C, THF, RT, 10 h, 97 % yield; (c) **9**, DCC, DPTS, THF, RT, 14 h, 97 % yield; (d) 50 atm H_2, Pd/C, THF, RT, 10 h, 94 % yield; (e) **9**, DCC, DPTS, THF, RT, 14 h, 94 % yield; (f) 50 atm H_2, Pd/C, THF, RT, 10 h, 95 % yield; (g) **9**, DCC, DPTS, THF, RT, 14 h, 90 % yield; (h) 50 atm H_2, Pd/C, THF:MeOH (9:1), RT, 10 h, 95 % yield; (i) pyridine, RT, 18 h, 95 % yield; (ii) TBDPSi-Cl, imidazole, DMF, RT, 48 h, 86 % yield; (iii) 50 psi H_2, 20 % $Pd(OH)_2$/C, THF, RT, 3 h, 95 % yield; (iv) **9**, DCC, DPTS, DCM, RT, 18 h, 88 % yield; (v) TBAF, THF, RT, 1 h, 87 % yield; (vi) **11**, DCC, DPTS, DCM, RT, 18 h, 83 % yield; (vii) TBAF, THF, RT, 1 h, 83 % yield; (viii) **5**, DCC, DPTS, DCM, RT, 72 h, 73 % yield; (ix) 50 psi H_2, 20 % $Pd(OH)_2$/C, THF, RT, 3 h, 97 % yield.

developed for the divergent method. Within these constraints, the *t*-butyldiphenylsilyl ester protecting group was identified as optimal. This protecting group is stable to esterification and hydrogenolysis conditions, while it can be selectively cleaved by tetrabutylammonium fluoride (TBAF) at room temperature.

The convergent synthesis begins by synthesizing the branching unit **11** that is used in subsequent generation growth coupling reactions. Compound **11** is obtained in two steps from 2-(*cis*-1,3-*O*-benzylidene glycerol)-succinic acid (**9**): first, by protecting the carboxyl group with *t*-butyldiphenylsilyl chloride followed by hydrogenolysis of the benzylidene acetal. The resulting monomer **11** has two primary alcohols available for functionalization and a protected focal point. Next, the second-generation dendron is synthesized by coupling 2 equiv of **9** with **11** using a similar DCC/DPTS esterification procedure as in the divergent approach. The focal point of this dendron is deprotected with TBAF and is then coupled with monomer **11** to form a third-generation dendron. Complete experimental details of the convergent synthesis of a G4 dendron and G3 dendrimer have been recently published [39].



14

Fig. 4 Symmetrical anhydride monomer.

The overall yields for each step in the divergent and convergent syntheses are compared in Table 1. In general, the divergent synthesis is advantageous for the preparation of dendrimers on a large scale, while the convergent approach is better suited for the preparation of more intricate dendritic macromolecules.

Table 1 Comparison of individual reaction and overall yields for the divergent and convergent synthesis of a third-generation PGLSA dendrimer. Note: Reaction letters and numbers are from Scheme 1.

Divergent product	Rxn.	Reaction yield	Overall yield	Convergent product	Rxn.	Reaction yield	Overall yield
[G1]-PGLSA-bzld	c	97 %	97 %	bzld-**[G2]-PGLSA**-TBDPS	iv	88 %	88 %
[G1]-PGLSA-OH; **6**	d	94 %	91 %	bzld-**[G2]-PGLSA**-acid; **12**	v	87 %	77 %
[G2]-PGLSA-bzld	e	94 %	86 %	bzld-**[G3]-PGLSA**-TBDPS	vi	83 %	64 %
[G2]-PGLSA-OH; **7**	f	95 %	81 %	bzld-**[G3]-PGLSA**-acid; **13**	vii	83 %	53 %
[G3]-PGLSA-bzld	g	90 %	73 %	**[G3]-PGLSA**-bzld	viii	73 %	39 %
[G3]-PGLSA-OH; **8**	h	95 %	70 %	**[G3]-PGLSA**-OH; **8**	ix	97 %	37 %

New materials and procedures are highly sought after to repair or regenerate damaged and diseased tissues. The use of degradable, biocompatible polymeric materials as temporary tissue scaffolds or sealants to assist healing are of interest for a variety of disciplines from orthopedics to ophthalmology. In the field of ophthalmology, alternatives to sutures would be highly desirable when repairing certain damaged tissues of the eye. For example, the cornea is the clear, avascular dome-shaped structure in the front of the eye that is responsible for focusing two-thirds of the light rays entering the eye. Because of its location, the cornea is particularly prone to traumatic injury, which usually leads to decreased vision. Current procedures for repairing corneal tissue involve suturing the injury site. This process is associated with several disadvantages including scarring, neovascularization, and wound distortion.

Repairing corneal lacerations and securing laser-assisted in situ keratomileusis (LASIK) flaps are two examples where a photocrosslinkable sealant would offer advantages over sutures. Corneal lacera-

tions or perforations are caused by trauma, infection, and inflammation and represent a common ophthalmic emergency with potentially blinding sequelae due to corneal scarring, astigmatism, and lens damage. LASIK is the popular refractive surgical procedure used to correct myopia (near-sightedness) and astigmatism. In this procedure, a thin, hinged corneal flap is created by a microkeratome blade and then moved aside to allow an eximer laser beam to ablate the corneal stromal tissue with extreme precision. Afterwards, the flap is then repositioned and allowed to self-seal. However, this flap can become dislocated prior to healing, resulting in flap striae (folds) and severe visual loss. When this complication occurs, treatment involves prompt replacement of the flap and, not infrequently, flap suturing.

Today, sutures are used to repair both corneal wounds and to secure LASIK flaps. In both of these cases, the pattern and extent of injury will determine if multiple sutures are needed to restore the structural integrity of the cornea. The process of suturing causes additional trauma to the corneal tissue and increases the risk of infection, inflammation, and vascularization. Also, sutures require postoperative removal, add the risk of becoming loose or breaking, and commonly induce astigmatism.

A degradable, photocurable biodendrimer sealant was prepared by reacting the (**[G1]-PGLSA**-OH)$_2$-**PEG**$_{3400}$ dendritic-linear polymer [35] with methacrylic anhydride to afford the methacrylated derivative. The resulting polymer can be subsequently photocrosslinked to form a hydrogel using visible light in conjunction with a photo initiating system [e.g., eosin Y (EY), triethanolamine (TEA), *N*-vinyl pyrrolidinone (VP)]. This system is capable of closing a wound or filling a defect. The first experiments using this novel sealant were performed on enucleated eyes to determine the strength of the secured wound. The PGLSA dendritic derivative allows for control over the rate of polymerization via irradiation time and, as a consequence, the rate of wound closure. In this corneal laceration study, a full-thickness 4.1-mm linear incision was made in the central cornea of 36 enucleated eyes. Twenty-seven of these eyes were repaired with the methacrylated biodendrimer adhesive, and nine were repaired using 10-0 monofilament nylon sutures. Prior to repair, all wounds were confirmed to be Seidel-positive (signifying a leak of aqueous fluid from the anterior chamber through the operative wound), indicating the wound cannot close by itself.

The biodendrimer adhesive (10 µl, (**[G1]-PGLSA**-MA)$_2$-**PEG**$_{3400}$, 30 % w/v aqueous solution) was applied to a 4.1-mm linear laceration in 27 eyes using a 30 gauge needle attached to a 1-cc syringe [58]. The adhesive was applied to the inner borders of the laceration as well as to the surface of the corneal laceration. Next, an argon ion laser (200 mW; 1 s pulse duration) was used to polymerize the biodendrimer and form a clear crosslinked gel that subsequently closed the wound. In contrast, the nine control eyes were closed using interrupted 10-0 nylon sutures.

A cardiac transducer was used to monitor the intraocular pressure (IOP) of all repaired eyes as reported in similar experiments described in the literature [59]. After the wound was secured with the biodendritic sealant or suture, a saline solution was slowly injected into the eye via a syringe pump while the IOP was measured by a transducer. The IOP was recorded when leaking from the wound was observed. The mean leakage pressure for the wounds receiving sutures was 78.7 mm Hg (standard deviation of 27.8 mm Hg). The minimum and maximum values were 20 mm Hg and 117 mm Hg, respectively. The mean leakage pressure for the wounds treated with dendrimer sealant was 109.6 mm Hg (standard deviation of 82.7 mm Hg). The minimum and maximum values were 16 mm Hg and 360 mm Hg, respectively. An IOP of 34 mm Hg is an approximate physiological upper-limit that occurs under stressful functions such as coughing, valsalva maneuver, or exercising. Importantly, the biodendrimer sealant was able to secure the wound under physiologically relevant IOP.

Additional corneal laceration studies were conducted in vivo on 28 white leghorn chicken eyes. A 4-mm full-thickness wound was created in the right eye of each animal. Fourteen chicken corneas were repaired with the corneal sealant (Fig. 5A), and 14 were repaired with 10-0 monofilament nylon (Fig. 5B). All wounds were confirmed to be Seidel-positive. Chickens were sacrificed on postoperative days one, three, and seven. The photocrosslinkable dendrimer sealant successfully sealed 100 % of the lacerations. All wounds sealed with the biodendrimer and nylon sutures were Seidel-negative after postoperative day one. However, the wounds receiving the polymer sealant appeared to have less corneal

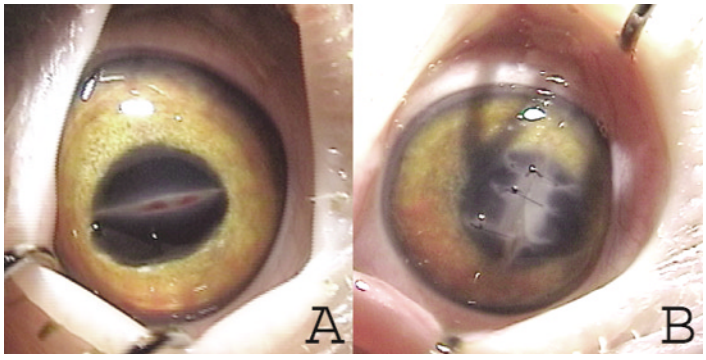


Fig. 5 Full-thickness corneal lacerations secured with the photocrosslinkable dendrimer sealant (A) or with a traditional suturing technique (B). Photographs of sealed wounds at 7days.

hazing and scarring compared to the suture group. More significantly, histological sections at day seven showed that the wounds receiving the biodendrimer sealant were histologically superior, possessing more uniform corneal structure than the suture-treated wounds. Additional animal studies are ongoing to follow the time-course of healing out to four weeks.

For the LASIK flap study, hinged corneal flaps were created using the Hansatome microkeratome system on four human donor eyebank eyes (Fig. 6A). Flap adherence was initially tested with dry Merocel sponges and tying forceps. This test showed that the corneal flaps were easily moveable with minimal force. The (**[G1]-PGLSA**-MA)$_2$-**PEG**$_{3400}$ biodendrimer tissue adhesive was then applied to the entire flap edge and polymerized with an argon laser beam. Flap adherence was retested after the dendrimer sealant was placed and crosslinked (Fig. 6B). The results confirmed that flap position was maintained despite considerable force placed on the flap. In addition, the crosslinked dendritic adhesive remained intact with no visible evidence of flap dehiscence. Although these tests are qualitative in nature, the strength and effectiveness of the biodendrimer adhesive in securing flap position and adherence was demonstrated. Overall, the ease of application of the dendrimer sealant and control in repairing the wounds suggests that these materials may prove to be superior to conventional suture treatments for a number of ophthalmic surgeries.

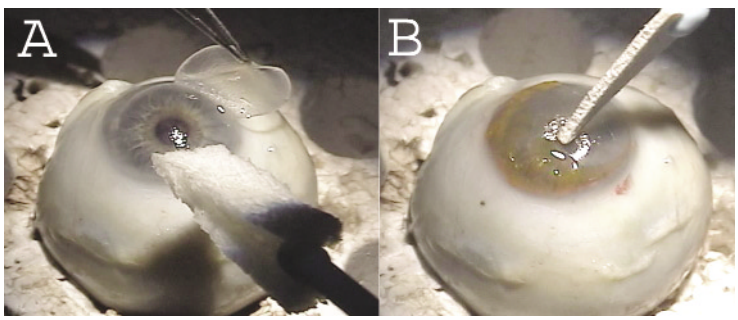


Fig. 6 Hinged corneal flaps were created using the Hansatome microkeratome system (A) and secured with photocrosslinked dendrimer sealant (B).

The recent success with dendritic polymers to seal corneal wounds has further motivated our research effort to design, synthesize, and evaluate polymers with specific properties (e.g., chemical, physical, optical, mechanical, and biological) for medical applications. Such chemically tailored macromolecules are likely to be of interest for drug delivery, gene transfection, and cartilage tissue engineering. Additionally, the versatility of the divergent and convergent synthetic methodologies de-

veloped allows for the preparation of a host of dendritic polymers and biomaterials. Finally, this interdisciplinary effort has afforded new synthetic routes and dendritic macromolecules while simultaneously addressing a clinically relevant problem with a novel solution.

ACKNOWLEDGMENTS

This work was supported by the NIH (R01 EY13881), the Pew Scholars Program in the Biomedical Sciences, and the Johnson and Johnson Focused Giving Program. We thank the North Carolina Eye Bank. MWG 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.

REFERENCES

1. A. W. Bosman, H. M. Janssen, E. W. Meijer. *Chem. Rev.* **99**, 1665–1688 (1999).
2. M. Fischer and F. Vögtle. *Angew. Chem., Int. Ed.* **38**, 884–905 (1999).
3. G. R. Newkome, C. N. Moorefield, F. Vögtle. *Dendritic Molecules: Concepts, Synthesis, Perspectives*, VCH, New York (1996).
4. D. A. Tomalia, A. M. Naylor, W. A. Goddard. *Angew. Chem., Int. Ed. Engl.* **29**, 138–175 (1990).
5. S. Gestermann, R. Hesse, B. Widndisch, F. Vögtle. *Dendritic Architectures*, Wiley-VCH, Weinheim (2000).
6. O. A. Mathews, A. N. Shipway, J. F. Stoddart. *Prog. Polym. Sci.* **23**, 1–56 (1998).
7. F. Zeng and S. C. Zimmerman. *Chem. Rev.* **97**, 1681–1712 (1997).
8. C. J. Hawker and M. Piotti. *Speciality Monomers and Polymers,* ACS Symposium Series **755**, pp. 107–118, American Chemical Society, Washington, DC (2000).
9. C. J. Hawker and J. M. J. Fréchet. *Step-Growth Polymers for High Performance Materials,* ACS Symposium Series **624**, pp. 132–144, American Chemical Society, Washington, DC (1996).
10. G. R. Newkome, C. N. Moorefield, J. N. Keith, G. R. Baker, G. H. Escamilla. *Angew. Chem., Int. Ed.* **33**, 666–668 (1994).
11. F. Vögtle, S. Gestermann, R. Hesse, H. Schwierz, H. Windisch. *Prog. Polym. Sci.* **25**, 987–1041 (2000).
12. R. F. Service. *Science* **267**, 458–459 (1995).
13. C. J. Hawker and J. M. J. Fréchet. *J. Am. Chem. Soc.* **112**, 7638–7647 (1990).
14. S. M. Grayson and J. M. J. Fréchet. *Chem. Rev.* **101**, 3819–3868 (2001).
15. J. M. J. Fréchet. *Science* **263**, 1710–1715 (1994).
16. D. A. Tomalia, H. Baker, J. Dewald, M. Hall, G. Kallos, S. Martin, J. Roeck, J. Ryder, P. Smith. *Macromolecules* **19**, 2466–2468 (1986).
17. J. Issberner, R. Moors, F. Vögtle. *Angew. Chem., Int. Ed. Engl.* **33**, 2413–2420 (1994).
18. W. Buhleier, F. V. Wehner, F. Vögtle. *Synthesis* 155–158 (1987).
19. M. J. Cloninger. *Curr. Opin. Chem. Biol.* **6**, 742–748 (2002).
20. R. Esfand and D. A. Tomalia. *Drug Discov. Today* **6**, 427–436 (2001).
21. M. Liu and J. M. J. Fréchet. *Pharm. Sci. Technol. Today* **2**, 393–401 (1999).
22. F. Aulenta, W. Hayes, S. Rannard. *Eur. Polym. J.* **39**, 1741–1771 (2003).
23. A. K. Patri, I. J. Majoros, J. R. Baker. *Curr. Opin. Chem. Biol.* **6**, 466–471 (2002).
24. E. C. Wiener, M. W. Brechbeil, H. Brothers, R. L. Magin, O. A. Gansow, D. A. Tomalia, P. C. Lauterbur. *Magn. Reson. Med.* **31**, 1–8 (1994).
25. N. Wimmer, R. J. Marano, P. S. Kearns, E. P. Rakoczy, I. Toth. *Bioorg. Med. Chem. Lett.* **12**, 2635–2637 (2002).
26. L. Qin, D. R. Pahud, Y. Ding, A. U. Bielinska, J. F. Kukowska-Latallo, J. R. Baker, Jr., J. S. Bromberg. *Hum. Gene Ther.* **9**, 553–560 (1998).

27. Superfect® product of Qiagen, Inc.
28. C. J. Hawker, K. L. Wooley, J. M. J. Fréchet. *J. Chem. Soc., Perkin Trans. I* 1287–1297 (1993).
29. J. F. G. A. Jansen, E. W. Meijer, E. M. M. de Brabander-van den Berg. *J. Am. Chem. Soc.* **117**, 4417–4418 (1995).
30. G. R. Newkome, B. D. Woosley, E. He, C. N. Moorefield, R. Guether, G. R. Baker, G. H. Escamilla, J. Merrill, H. Luftmann. *Chem. Comm.* **24**, 2737–2738 (1996).
31. M. T. Morgan, M. A. Carnahan, C. E. Immoos, A. A. Ribeiro, S. Finkelstein, S. J. Lee, M. W. Grinstaff. *J. Am. Chem. Soc.* **125**, 15485–15489 (2003).
32. K. Kono, M. Liu, J. M. J. Fréchet. *Bioconjugate Chem.* **10**, 1115–1121 (1999).
33. A. Quintana, E. Raczka, L. Piehler, I. Lee, A. Myc, I. Majoros, A. K. Patri, T. Thomas, J. Mule, J. R. J. Baker. *Pharm. Res.* **19**, 1310–1316 (2002).
34. M. W. Grinstaff. *Chem. Eur. J.* **8**, 2838–2846 (2002).
35. M. A. Carnahan, C. Middleton, J. Kim, T. Kim, M. W. Grinstaff. *J. Am. Chem. Soc.* **124**, 5291–5293 (2002).
36. <www.starpharma.com>
37. M. A. Carnahan and M. W. Grinstaff. *J. Am. Chem. Soc.* **123**, 2905–2906 (2001).
38. M. A. Carnahan and M. W. Grinstaff. *Macromolecules* **34**, 7648–7655 (2001).
39. N. R. Luman, K. A. Smeds, M. W. Grinstaff. *Chem. Eur. J.* **9**, 5618–5626 (2003).
40. S. H. M. Sontjens, D. L. Nettles, M. A. Carnahan, L. A. Setton, M. W. Grinstaff. *International Tissue Engineering Meeting*, Orlando, FL, ABS# 21.4 (2003).
41. T. Kim. *Vision Pan-America* **3**, 4–6 (2003).
42. J. C. Kern. *Encyclopedia of Chemical Technology*, M. Grayson (Ed.), John Wiley, New York (1984).
43. C. K. Mathews, K. E. van Holde, K. G. Ahern. *Biochemistry*, 3rd ed., Addison Wesley Longman, San Francisco (2000).
44. L. O. Winstrom. *Encyclopedia of Chemical Technology*, M. Grayson (Ed.), John Wiley, New York (1984).
45. P. H. J. Carlsen, K. Sorbye, T. Ulven, K. Aasbo. *Acta Chim. Scand.* **50**, 185–187 (1996).
46. T. W. Greene and P. G. M. Wuts. *Protective Groups in Organic Chemistry*, John Wiley, New York (1991).
47. S. Hanessian, T. S. Liak, B. Vanasse. *Synthesis* **5**, 396–397 (1981).
48. S. Li and M. Vert. *Degradable Polymers*, 2nd ed., pp. 71–131, Kluwer Academic, Dordrecht (2002).
49. N. Yui and T. Ooya. *Supramolecular Design for Biological Applications*, 169–189 (2002).
50. S. J. Huang. *Degradable Polymers*, 2nd ed., pp. 17–26, Kluwer Academic, Dordrecht (2002).
51. K. A. Athanasiou, G. G. Niederauer, A. C. M. Agrawal. *Biomaterials* **17**, 93–102 (1996).
52. N. Ashammakhi and P. Rokkanen. *Biomaterials* **18**, 3–9 (1997).
53. R. E. Drumright, P. R. Gruber, D. E. Henton. *Adv. Mater.* **12**, 1841–1846 (2000).
54. D. A. Barrera, E. Zylstra, P. T. Lansbury, R. Langer. *J. Am. Chem. Soc.* **115**, 11010–11011 (1993).
55. J. Elisseeff, K. Anseth, R. Langer, J. S. Hrkach. *Macromolecules* **30**, 2182–2184 (1997).
56. H. Ihre, O. L. P. De Jesus, J. M. J. Fréchet. *J. Am. Chem. Soc.* **123**, 5908–5917 (2001).
57. F. Vögtle. *Dendrimers II: Architecture, Nanostructure, and Supramolecular Chemistry*, Springer, Berlin (2000).
58. A. J. Velazquez, M. A. Carnahan, J. Kristinsson, S. Stinnett, M. W. Grinstaff, T. Kim. *Arch. Ophthalmol.* **122**, 867–870 (2004).
59. D. O. Magnante, J. D. Bullock, R. Green. *Ophthalmology* **104**, 608–615 (1997).