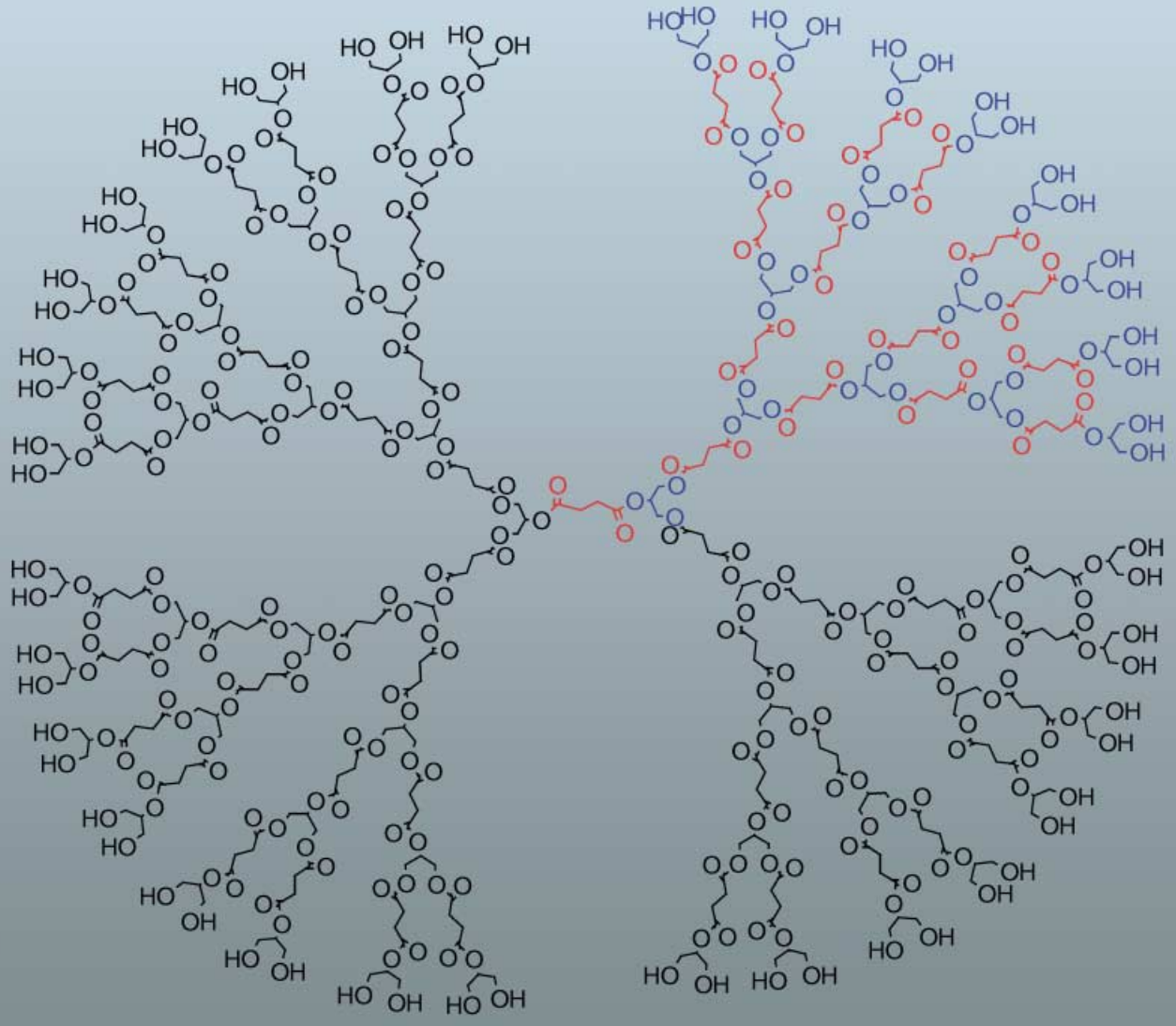
Biodendrimers: New Polymeric Biomaterials for Tissue Engineering



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Biodendrimers: New Polymeric Biomaterials for Tissue Engineering

Mark W. Grinstaff*[a]

Abstract: Dendrimers composed entirely of building blocks known to be biocompatible or degradable in vivo to natural metabolites were synthesized using a high yield divergent approach. This synthesis is amenable to the design and development of new biomaterials that are of interest for a variety of medical applications, including tissue engineering. In a novel application, photocrosslinkable dendrimers are used to seal large corneal lacerations.

Keywords: chemical biology · dendrimers · gels · materials science · polymers

Introduction

Advances in polymer chemistry and engineering have contributed to many of the industrial and medical achievements over the past 50 years. In the medical and biotechnology areas, polymers are components of a variety of medical devices ranging from the common catheter to functional prostheses. Today linear polymers are the macromolecules of most widespread use. However, other polymer architectures are available, for example, block, branch, comb, and especially dendritic. Dendritic macromolecules offer a myriad of possibilities for designing unique structures with specific properties.

Dendrimers are monodisperse polymers that adopt a globular three-dimensional shape as the generation number (Gn) increases.[1–22] These highly-branched macromolecules possess a well-defined core, interior region layers, and an exterior corrugated surface affording a high surface-area-to-volume ratio. With each successive generation, the number of end groups doubles and the properties of the dendrimer become more strongly influenced by the nature of the end group. These chemical and structural attributes of dendrimers translate to unique chemical and physical properties (e.g., solubility, chemical reactivity, viscosity, glass transition temperature). Thus, opportunities exist to control both the three-dimensional structure and material properties of dendrimers through specific alterations at the molecular level. Since the first dendrimer synthesis described by Vögtle,[23] these macromolecules have been actively investigated for a wide range of applications including catalysis,[24–34] molecular encapsulation,[35–44] light harvesting,[45–53] and drug delivery.[9, 11, 54–66]

Our working concept is that dendrimers composed of natural metabolites or biocompatible building blocks will possess unique chemical, physical, and mechanical properties, and these new biomaterials will provide tailored solutions to current tissue engineering challenges.[67–72] We are presently developing new polymer synthesis methodologies, synthesizing dendritic polymers, and incorporating heteroatom groups in the polymer structure for further derivatization. For each new polymer, we characterize the macromolecule by NMR, SEC, MALDI and DSC, determine the kinetics and mechanism of polymer degradation, evaluate the rheological properties, and measure the mechanical properties of the crosslinked dendritic assemblies. In addition, we are evaluating these dendritic macromolecules as injectable tissue adhesives and temporary cell scaffolds as a function of molecular composition, structure, and properties. We have synthesized a number of new dendritic macromolecules and have evaluated one series of dendritic polymers for effectiveness in repairing large corneal lacerations.

Monomer Selection

To optimize the chemical and physical properties (e.g., solubility, viscosity, adhesiveness, degradability, and in vivo resident time and response) of a dendrimer, it is crucial to identify the appropriate monomer building block(s). The dendrimers described in the present article are composed of biocompatible monomers, and hence termed "biodendrimers."[73] Suitable candidates for biodendrimer synthesis include natural metabolites, chemical intermediates found in metabolic pathways, and monomers currently used for the preparation of medical-grade linear polymers.

Monomers that are natural metabolites include amino acids, sugars, α-hydroxy acids, and fatty acids. The amino acids represent a large class of suitable monomers. In 1984 a poly(lysine) dendrimer was first described.[74] and since

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several peptide-based dendrimers have been described.[75–77] Later in that decade, Tam reported the use of poly(lysine) dendrimers as multiple antigen peptides.[78–80] More recently, Zimmerman has prepared peptide dendrimers using a cyanoethylation–hydrogenation strategy.[81] Naturally occurring α-hydroxy acids are building blocks of synthetic linear polyesters such as poly(lactic acid) (PLA) and poly(glycolic acid) (PGA).[82, 83] PLA was first used as a bioresorbable surgical suture,[84] and poly(hydroxy acids) are presently being widely investigated for drug delivery (e.g., microspheres, hollow fibers), wound closure (e.g., staples, dressings, meshes), and orthopaedic applications (e.g., screws, pins, scaffolds).

Chemical intermediates found in metabolic pathways include, for example, succinic acid, fumaric acid, citric acid, and pyruvic acid. Monomers found in clinically used synthetic polymers (e.g. poly(ethylene glycol) (PEG), poly(caprolactone) (PCL), poly(trimethylene carbonate) (TMC), etc.) are also prime candidates. Incorporation of one or more of these monomer building blocks in a dendritic structure[73, 85] provides new opportunities to create well-defined, tailored polymers for medical applications.[67–71, 86, 87]

Syntheses

Several synthetic approaches are currently used to prepare aromatic and aliphatic dendritic macromolecules: divergent (from core to surface),[13, 18–20, 88, 89] convergent (from surface to core),[17, 90–94] and double-stage convergent.[92, 95] In a recent *Chemical Reviews* article, Fréchet provides a detailed analysis and description of the convergent and double-stage convergent methods for dendrimer synthesis.[96] The divergent approach was reported in the mid 1980's,[19, 20] and several thorough reviews on this topic are described by Newkome and Vögtle.[7, 97, 98]

Presently, we are using the divergent approach to synthesize biodendrimers constructed from a branching AB_2 monomer unit possessing one carboxylic acid and two hydroxyl groups. This approach is described in the recent literature for the synthesis of polyether-ester and polyester biodendrimers composed of glycerol and lactic acid or succinic acid, respectively.[73, 85]

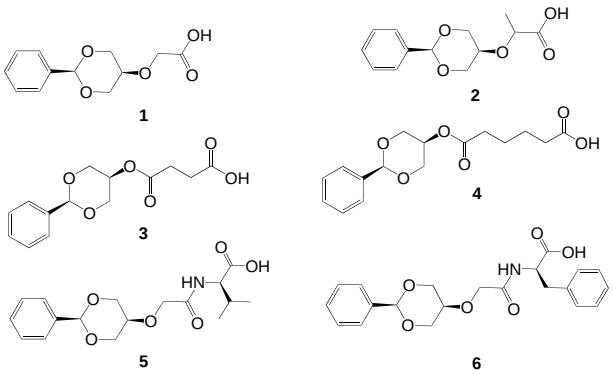
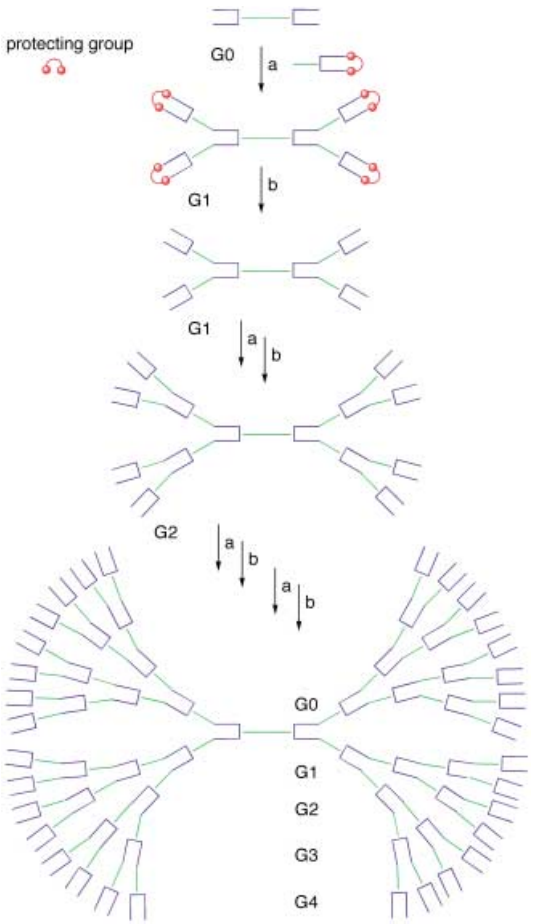


Figure 1. Examples of monomers being explored for biodendrimer synthesis.

Scheme 1 depicts the divergent synthesis of a generation four (G4) dendrimer. The dendritic architecture is created by coupling the monomer in a head-to-tail manner. This divergent approach requires the use of a protecting group for the tail; each generation, synthesized iteratively, requires a coupling followed by a deprotection reaction.



Scheme 1. The divergent synthesis of dendrimers. Coupling of the monomer in a head (blue) to tail (green) manner creates the dendritic architecture. This divergent approach requires the use of a protecting group (red) which facilitates a step-by-step process of coupling followed by deprotection to create larger and larger dendrimers. a) coupling; b) deprotection.

A representative list of the AB_2 monomers we are investigating for biodendrimer synthesis is shown in Figure 1. Monomers **1** and **2** are adducts of glycerol and glycolic acid or lactic acid, respectively, whereas monomers **3** and **4** are derivatives of succinic and adipic acid. Monomer **3** and **4**, unlike the first two, will afford a polyester dendrimer as opposed to polyether-ester dendrimers. Additionally, the hydrophobicity of the branching unit increases from monomer **1** to **4**. Monomers **5** and **6** are glycerol–glycolic acid amino acid derivatives, where the hydrophilicity/hydrophobicity of the monomer can be tuned by selecting the appropriate amino acid. In all of these monomers, glycerol is the key molecular structure constituting the branching unit required for the dendritic architecture. The 1,3-hydroxyl groups of glycerol are protected with a benzylidene acetal

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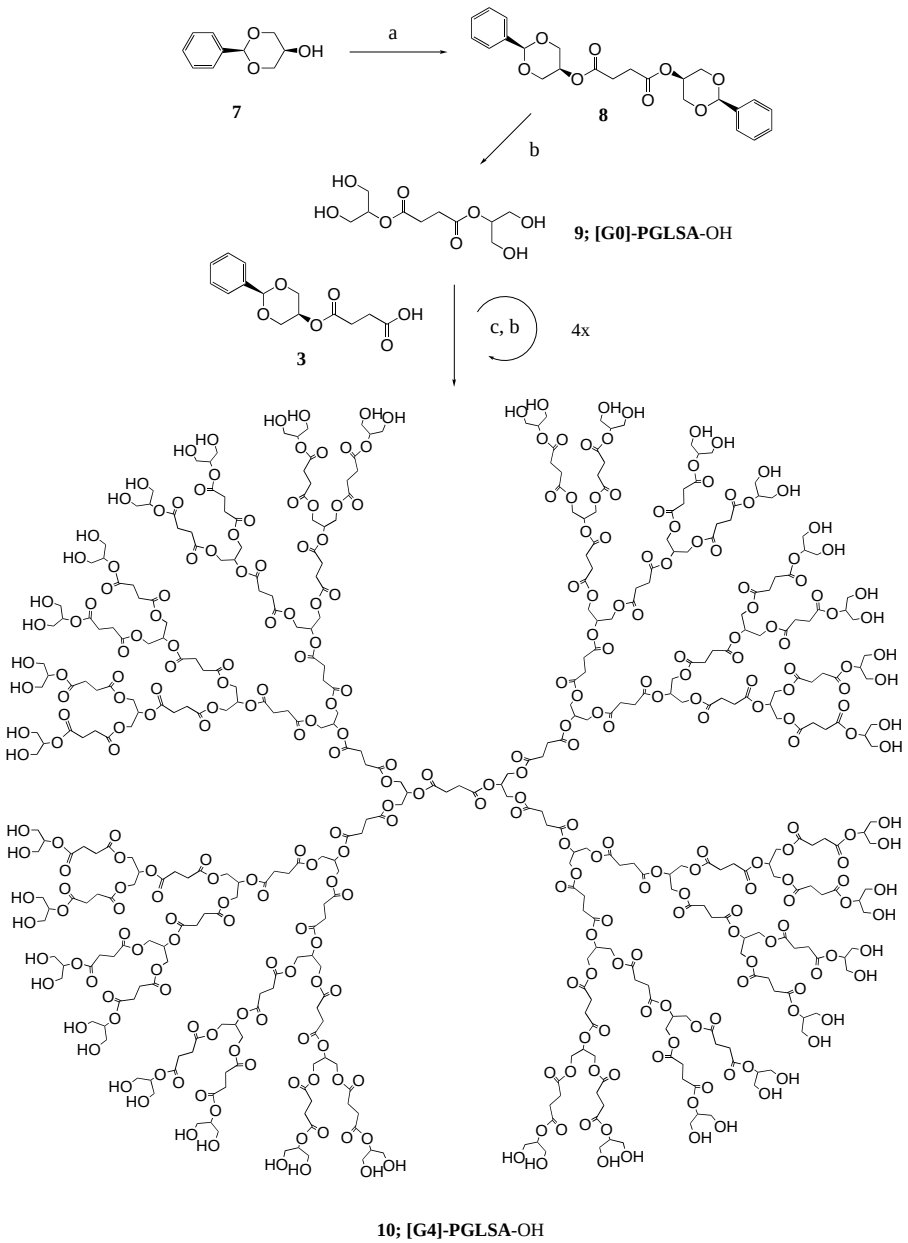
group (bzld), which is selectively cleaved under neutral conditions by hydrogenolysis (Pd/C or $Pd(OH)_2$/C and H_2). Moreover, the bzld group ensures sufficient solubility in common organic solvents for dendrimer purification and serves as an NMR tag for monitoring the successive dendrimer generation reactions.

This approach is exemplified in the synthesis of a generation four *p*oly(*gl*ycerol-*s*uccinic *a*cid) dendrimer, **[G4]-PGLSA**-OH (Scheme 2). First, a tetra-functional G0 core, **9**, was synthesized in two steps.[73, 85] Succinic acid was coupled to two equivalents of *cis*-1,3-*O*-benzylideneglycerol (**7**) in the presence of three equivalents of *N*,*N*-dicyclohexylcarbodiimide (DCC) and one equivalent of 4-(dimethylamino)pyridinium 4-toluenesulfonate (DPTS) to yield **8** (90% yield). The blzd group of the core was removed by hydrogenolysis (10% Pd/C, 50 psi H_2; THF) to afford the tetrahydroxy core, **[G0]-PGLSA**-OH, **9**, (97%). The branching ligand, **3** (2-(*cis*-1,3-*O*-benzylidene-glycerol)succinic acid mono ester), was prepared by treating **7** with succinic anhydride in pyridine (95% yield). Next, compounds **3** and **9** were coupled in the presence of DCC and DPTS to afford the protected G1 dendrimer. Successive iteration of the esterification reaction followed by the hydrogenolysis reaction afforded the larger G2 through G4 **PGLSA** dendrimers. All the reaction yields were good to excellent, and the overall reaction yield from G0 to the **[G4]-PGLSA**-OH dendrimer, which included ten steps, was 41%.

The esterification and deprotection reactions were monitored by ^{1}H NMR spectroscopy since the relative integrated areas of the aromatic, glycerol, and succinic acid protons change with each successive generation. Molecular weight data were determined by FAB/MALDI-TOF and size-exclusion chromatography (SEC). The polydispersity indices (PDIs) were 1.02 for all of the **PGLSA** dendrimers. As expected, the agreement between the calculated molecular weight and the SEC determined molecular weight was high, but worsened as the generation number increased. The molecular weight of the **[G4]-PGLSA**-OH dendrimer is 10715. The protected **PGLSA** dendrimers are amorphous solids at room temperature, whereas the deprotected **PGLSA** dendrimers are viscous liquids. The protected **PGLSA** dendrimers are soluble in common organic solvents (e.g., CH_2Cl_2, THF) and the hydroxyl-terminated **PGLSA** dendrimers are soluble in polar solvents such as dimethylformamide, ethanol, methanol, and water.

We have used the divergent approach to prepare more than 40 dendritic macromolecules in the laboratory. Figure 2 shows four representative examples including: a G1 *p*oly(*gl*ycerol-*s*uccinic *a*cid) polyester dendrimer, **[G1]-PGLSA**-OH,[85] a G2 *p*oly(*gl*ycerol-*g*lycolic *a*cid-*phe*nylalanine) polyester-amide, **[G2]-PGLGA-Phe**-OH,[99] a G3 *p*oly(*gl*ycerol-*l*actic *a*cid) polyester-ether dendrimer, **[G3]-PGLLA**-OH,[73] and a G4 *p*oly(*gl*ycerol-*s*uccinic *a*cid)-*p*oly(*e*thylene *g*lycol) polyester-ether hybrid dendritic-linear polymer, (**[G4]-PGLSA**-OH)$_2$-**PEG**.[100]



Scheme 2. The divergent synthesis of a **[G4]-PGLSA**-OH biodendrimer. a) succinic acid, DPTS, DCC, CH_2Cl_2, 25°C, 14h; b) 50 psi H_2, $Pd(OH)_2$/C, THF, 25°C, 10h; c) **3**, DPTS, DCC, THF, 25°C, 14h.

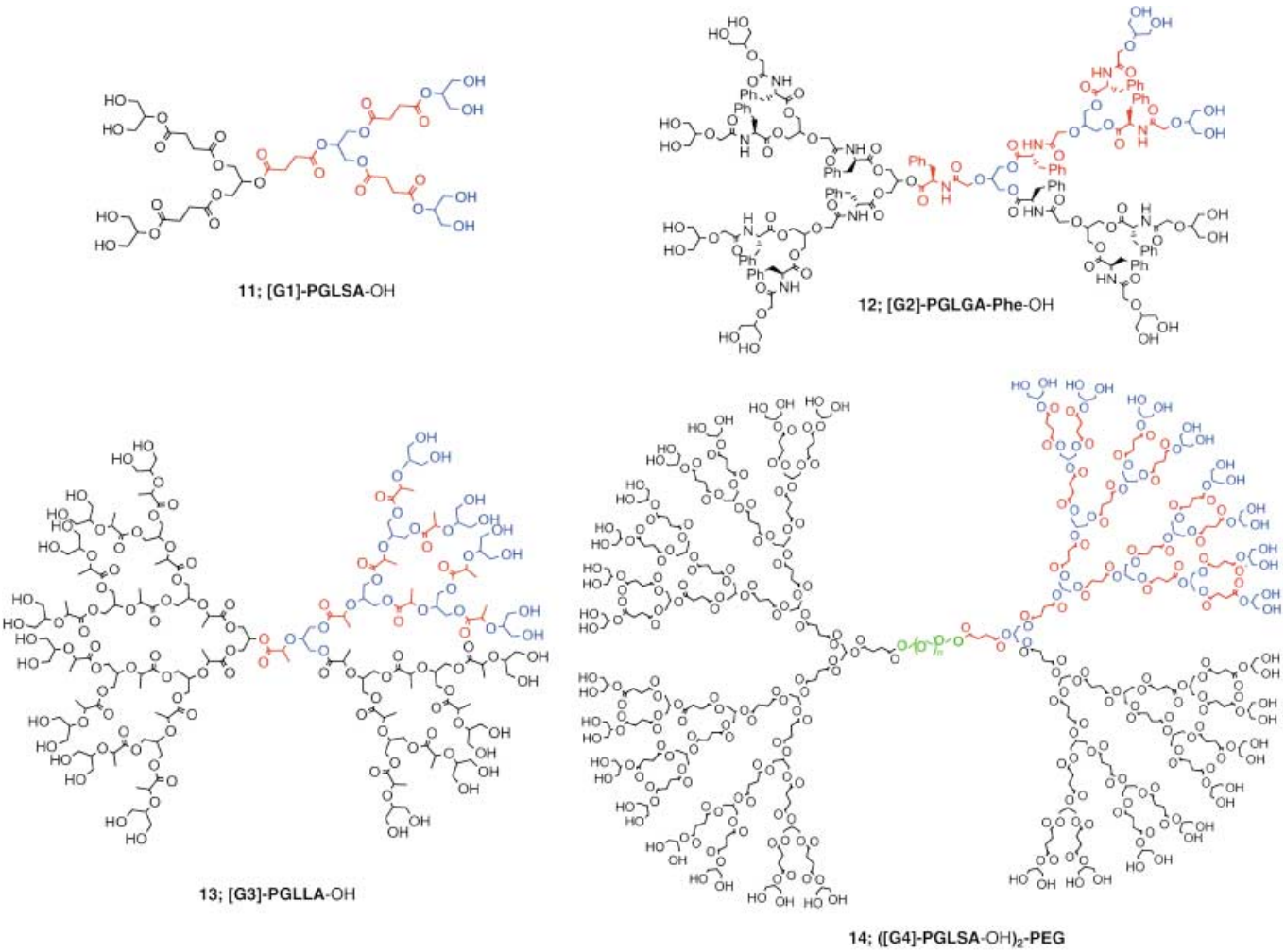


Figure 2. Representative examples of biodendrimers synthesized in the laboratory. blue = glycerol; red = succinic acid, phenylalanine, or lactic acid; green = PEG.

Degradation Characteristics

These dendritic macromolecules are designed to degrade in vivo. Consequently, tuning the degradation rate or altering the degradation mechanism of these materials is critical for future in vivo use. The dendrimers described here are susceptible to both acid/base and enzymatic degradation. The degradation rate can be controlled through four parameters: 1) The chemical bond between the monomer units. For example, ester bonds will be more susceptible to hydrolysis than ether or amide bonds. 2) The hydrophobicity of the monomer unit. Based on known degradation characteristics of linear polymers, a glycolic acid based dendrimer is likely to degrade faster than a corresponding caproic acid based dendrimer. 3) The generation number and molecular weight of the dendrimer. A larger more globular dendrimer is likely to degrade more slowly than a first-generation dendrimer. 4) The inherent chemical reactivity present in the macromolecule. A structural motif that favors an internal self-degradation reaction will accelerate the degradation rate. For example, we observed that PGLLA dendrimers degrade faster than PGLSA dendrimers. We attribute this reactivity difference to the facile loss of a monomer unit from a favorable internal six member cyclization reaction in the PGLLA dendrimers. Once the guidelines for altering the degradation rates of the biodendrimers are established, we can optimize the materials for a specific in vivo use, since the desired degradation rate of a synthetic biomaterial depends on the implantation site.

Tissue Engineering

The field of tissue engineering aspires to supplement or replace damaged or diseased tissue with functional synthetic constructs.[67–72] These biomaterial constructs ideally must be able to: 1) provide a temporary matrix for cells until a native extracellular matrix is formed, 2) direct the arrangement of different cells types to specific locations within the matrix, 3) modulate the biological activity through presentation of specific signaling molecules in an appropriate temporal and spatial manner, and 4) possess physical and mechanical properties reminiscent of the host native tissue site being replaced. At present, the polymers most used in clinical practice and investigated in basic science laboratories are linear polymers such as poly(lactic acid) (PLA), poly(glycolic

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acid) (PGA), and poly(ethylene glycol) (PEG). Although they are widely used, linear polymers suffer from an ill-defined solution structure, a large distribution of molecule weight, and limited control of polymer modification with one or more ligands.

Dendrimers may offer more promise in the medical arena. These macromolecules are currently being explored as vehicles for drug delivery,[9, 54–59] cationic agents for gene transfection,[60–64] inhibitors of influenza viruses,[65, 101–103] carriers for boron neutron capture therapy,[11, 104] and contrast agents for magnetic resonance imaging.[66] We are presently investigating dendritic macromolecules as new surgical closure materials for use in ophthalmology.

Specifically, we are synthesizing degradable, photocrosslinkable biodendrimer derivatives for repairing corneal wounds. Corneal lacerations and perforations arise from a variety of conditions including trauma, infection, and inflammation. Such corneal wounds can lead to corneal scarring, infection, cataract formation, synechiae formation, glaucoma, and blindness.[105–112] If treated quickly and effectively, many of these complications can be avoided.

In humans, corneal lacerations and perforations are most frequently repaired with sutures. However, the use of sutures has several drawbacks and limitations.[113, 114] First, the act of suturing inflicts additional trauma to corneal tissues affording an extended healing time. Second, nylon sutures can incite corneal inflammation and vascularization. Third, corneal suturing often leads to a regular or irregular astigmatism. Fourth, sutures may become loose and/or broken requiring subsequent postoperative care. Finally, suturing requires a high technical skill level.

An attractive alternative is a sutureless procedure using a tissue adhesive. Such a procedure immediately restores the integrity of the eye globe and decreases the risk of surgical complications.[115] In 1968, Webster et al. reported the use of cyanoacrylate glue for the repair of perforated corneal ulcers.[116] Although cyanoacrylate adhesives have proven to be an effective therapeutic option in certain ophthalmic settings such as sealing small corneal perforations (1 mm) and prophylactic treatment of progressive corneal thinning disorders,[116–122] they nevertheless have limitations with regard to their ease of applicability and effectiveness.[120, 123–130]

In our laboratory we are exploring corneal tissue repair through a new experimental strategy: in situ photocrosslinking of a polymer. This in situ photocrosslinking approach entails delivery of a liquid polymer followed by solidification of the polymer to form a three-dimensional polymeric network.[131] During this liquid to solid-phase transformation the corneal wound is sealed. The manipulation of the mechanical, electrical, or optical properties a synthetic construct or implant in vivo is an exciting technology being explored by several research groups, including ours.[69, 115, 132–137]

An effective polymer adhesive for repairing corneal perforations must meet a number of requirements. Ideally, it should 1) adhere to moist corneal surfaces, 2) possess rheological properties that allow for controlled and rapid placement of the polymer on the wound, 3) polymerize to seal the corneal wound only upon action of an external trigger (e.g., light), 4) quickly restore the intraocular pressure (IOP), 5) maintain the structural integrity of the eye, 6) possess a refractive index matching the native cornea, 7) possess solute diffusion properties favorable for normal corneal healing, 8) be biocompatible, and 9) be resorbed or extruded from the wound on a time scale consistent with tissue regeneration. Given these conditions, we hypothesized that the chemical control associated with dendrimers may provide an opportunity to design, synthesize, characterize, test, and optimize a dendritic macromolecule as an ophthalmic tissue adhesive.

To date, we have explored photocrosslinkable derivatives of the PGLSA[85] and PGLSA-PEG[100] dendritic macromolecules functionalized with methacrylate groups. After our initial studies, the hybrid dendritic–linear copolymers composed of succinic acid, glycerol, and poly(ethylene glycol) (**([Gn]-PGLSA)$_2$-PEG**) were selected for further investigation. We initiated an in vitro study to determine and compare the leaking pressure of corneal perforations sealed with standard sutures or the dendritic tissue adhesive.[100]

In determining the leaking pressure, first a 4.1 mm laceration was made in an enucleated eye with a keratome blade. The wound was then closed using either the photocrosslinkable biodendritic copolymer or three interrupted 10-0 nylon sutures. In the polymer repairing procedure, 10 μL of the methacrylated **([G0]-PGLSA)$_2$-PEG**, **([G1]-PGLSA)$_2$-PEG**, **([G2]-PGLSA)$_2$-PEG**, or **([G3]-PGLSA)$_2$-PEG** copolymer was applied to the laceration. Argon ion laser irradiation produced the dendritic gel sealing the wound (Figure 3; 200 mW, 1 s exposures; 50 s total irradiation time; the photoinitiator and co-catalyst used were ethyl eosin and TEA). Once sealed, we injected saline into the anterior chamber using a syringe inserted through the scleral until the repaired wound leaked. The leaking pressure for both the nylon suture ($N = 6$) and biodendrimer sealant ($N = 3$ for each copolymer tested) treated eyes was measured using a cardiac transducer probe inserted in the eye through the optic nerve. The mean leaking pressures (LP) for the sutured treated eyes was 90 ± 18 mm Hg. The leaking pressure for the eyes sealed with the G1 photocrosslinkable derivative **([G1]-PGLSA**-MA)$_2$**-PEG** (MA = methacrylate) was 171 ± 44 mm Hg. (For reference, normal intraocular pressure in a human eye is between 15 and 20 mm Hg.) The **([G0]-PGLSA**-MA)$_2$**-PEG** copolymer did not seal the wound and **([G2]-PGLSA**-MA)$_2$**-PEG** polymerized too quickly under the operating microscope to be delivered to the wound in a controlled fashion (LP < 15 mm Hg, for both copolymers). Copolymer **([G3]-PGLSA**-MA)$_2$**-PEG** was insoluble in water and only slightly soluble in alcohols, and when applied to the laceration did not seal the wound.

The **([G1]-PGLSA**-MA)$_2$**-PEG** hybrid linear–dendritic copolymer secures the corneal laceration better than conventional sutures. The repair procedure is approximately five times faster than suturing the wound and does not induce additional trauma to the wound. The crosslinked biodendritic gel is adhesive, elastic, and transparent. These are favorable properties for an ophthalmic sealant. The proposed mechanism responsible for sealing the wound with the hybrid dendritic–linear copolymer is formation of an interpenetrat-

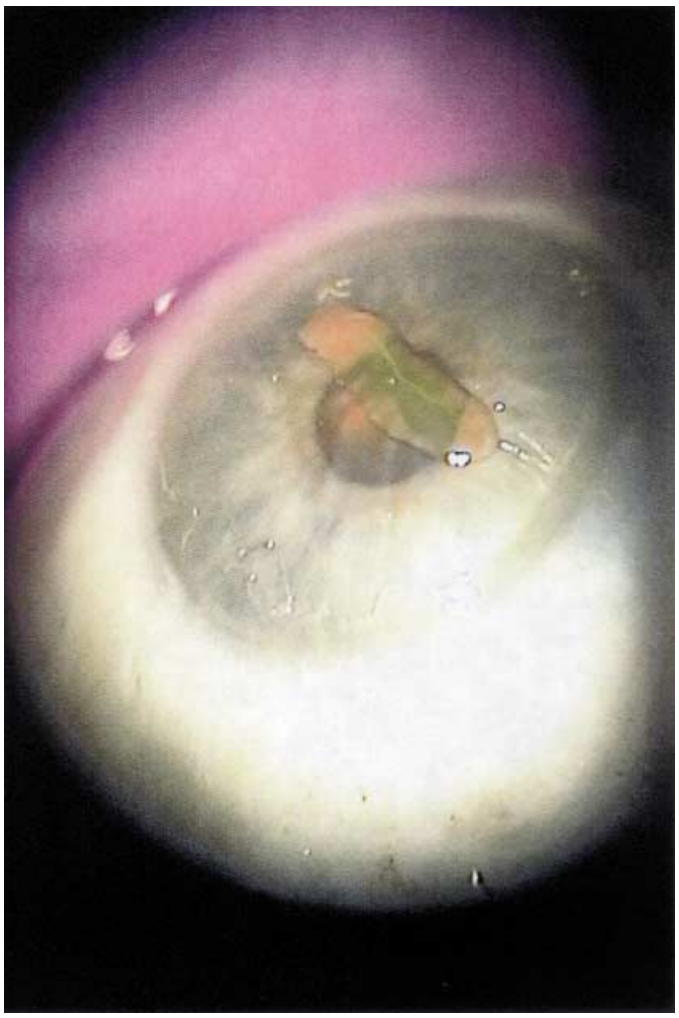

Figure 3. An optical micrograph of a sealed 4.1 mm corneal laceration using a photoactive PGLSA-PEG biodendrimer.

ing network (IPN) between the crosslinked copolymer and the tissue, that is, physical entrapment (see Figure 4). This

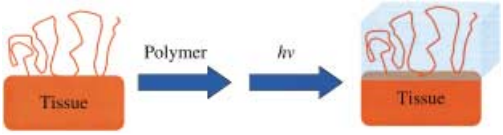

Figure 4. An illustration of the proposed IPN tissue sealing mechanism.

mechanism is substantiated by the observation that the copolymer alone without laser polymerization does not seal the wound. If the copolymer is too hydrophobic an IPN cannot form and tissue sealing does not occur.

Conclusion

In summary, the high yield divergent syntheses of dendrimers composed of glycerol, lactic acid, glycolic acid, phenylalanine, succinic acid, and poly(ethylene glycol) are described. This modular synthetic approach allows for control of the chemical and physical properties through discrete molecular changes at the core, interior, and/or exterior regions. Modification or functionalization of dendrimers for specific molecular interactions, biological signaling, or three-dimensional network formation through incorporation of photocrosslinking agents or ionic crosslinking agents, ligands for biological receptors, or pharmaceutical agents is an area actively studied by many laboratories. Functional biomaterials such as the photocrosslinkable biodendrimers are of interest for in situ photopolymerization where controlled and rapid photo-induced gel formation can occur in vivo. Using this procedure, we have successfully sealed experimental corneal lacerations, and are exploring the use of these materials for additional ophthalmic surgeries. Biodendrimers are novel macromolecules for medical applications (e.g., tissue engineering, drug delivery, sensors) that offer additional opportunities for molecular control of physical and chemical properties over conventional linear polymers.

This concept article is intended to briefly summarize our new results, pique scientific interest, and stimulate critical discussions. Moreover, it is an invitation to the general scientific community to examine this exciting area of macromolecular science that exists at the interface between chemistry and biology.

Acknowledgement

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