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In Situ Polymerized Hydrogels for Repairing Scleral Incisions Used in Pars Plana Vitrectomy Procedures**

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Crosslinked polymer networks possessing a high water content, otherwise known as hydrogels, are multipurpose materials for medical applications in areas such as drug delivery, tissue engineering, and wound healing.[1–7] To form such hydrogels, dendritic or highly branched macromers are advantageous.[8–15] These macromers provide opportunities to create hydrogels at low polymer concentration, to control swelling, and to vary the hydrogel mechanical properties. These favorable attributes arise with dendritic polymers because of the well-defined composition, the large number of endgroups, the physiochemical properties (such as low viscosity), and the preparation methods that allow for precise structural control and optimization capabilities. We are synthesizing dendritic macromolecules composed of biocompatible building blocks and evaluating the corresponding hydrogels as ocular adhesives.[16–19] Using a photocurable hydrogel system based on poly(glycerol-succinic acid)-polyethylene glycol hybrid dendritic-linear macromolecules, full-thickness 4.1 mm corneal lacerations in enucleated eyes and chicken eyes in vivo, as well as secured LASIK flaps in vitro, have been successfully repaired.[19–22] In addition to light-activated hydrogel formation, other crosslinking strategies that quickly afford a hydrogel adhesive are also explored.[22,23] Herein, we report the synthesis of lysine-terminated peptide dendrimers and dendrons, the formation of crosslinked hydrogels with a poly(ethylene glycol) di-activated ester, the analysis of hydrogel mechanical properties, and the closure of a sclerotomy incision—the wound created during a typical vitrectomy procedure.

The dendrons ([G1]-Lys-NH_2 and [G2]-Lys-NH_2) and dendrimers (([G1]-Lys-NH_2)$_2$-PEG and ([G2]-Lys-NH_2)$_2$-PEG) used for hydrogel formation were synthesized as shown in Scheme 1. Several amide-coupling approaches were explored (BOP, DCC, EDC, and oxalyl chloride), and the pentafluorophenol-ester strategy was found to yield the highest amide coupling reactions. Thus, we first prepared the pentafluorophenol-ester of ZLys(Z)OH and BocLys(Boc)OH using *N-N'*-dicyclohexylcarbodiimide (DCC) and 2,3,4,5,6-pentafluorophenol (PFP) in CH_2Cl_2. After crystallization in CH_2Cl_2/hexane, white crystalline products were obtained (98% and 92% yield, respectively). Next, ZLys(Z)OPFP was coupled to LysOMe·2HCl in the presence of diisopropylethylamine (DIEA) and 1-hydroxybenzotriazole (HOBT) to prevent racemization, to give **1**. Compound **1** was purified by precipitation in ether and obtained in 98% yield. The Z-amino protecting groups were cleaved by hydrogenolysis (Pd/C, H_2) in methanol (99% yield) and the amine functionality was subsequently acidified using 1 M HCl (99% yield) to afford the [G1]-Lys-NH_2 dendron, **2**. The larger dendron [G2]-Lys-NH_2, **3**, was synthesized by reacting **2** with BocLys(Boc)PFP in the presence of HOBT and DIEA, followed by treatment with TFA to remove the Boc protecting groups (70% and 99% yield, respectively). The protected intermediate was purified by silica gel chromatography (CH_2Cl_2/MeOH = 95:5). The corresponding product obtained using ZLys(Z)OPFP was difficult to isolate and purify. The intermediates prepared in the dendron synthesis were also used to prepare the ([G1]-Lys-NH_2)$_2$-PEG, **4** and ([G2]-Lys-NH_2)$_2$-PEG **5** dendrimers. ZLys(Z)OPFP was coupled to diamino polyethylene glycol [M_W = 3400] in the presence of DIEA and HOBT in CH_2Cl_2, to afford ([G1]-Lys-NH_2)$_2$-PEG **4**. The product was washed with water and purified by precipitation in ether (95% yield). The deprotection of the Z groups was achieved by hydrogenolysis (Pd/C, H_2) in methanol followed by precipitation in ether to afford ([G1]-Lys-NH_2)$_2$-PEG **4** as a white powder (99% yield). The previously prepared, protected dendron **1** was used for the synthesis of the ([G2]-Lys-NH_2)$_2$-PEG dendrimer, **5**. The methyl ester of **1** was hydrolyzed by saponification with 1 M NaOH in methanol followed by neutralization with 1 M HCl (85% yield). The pentafluorophenol-ester of this acid was obtained by treatment with 2,3,4,5,6-pentafluorophenol and DCC in CH_2Cl_2 to afford a white powder after crystallization (95% yield). This activated ester dendron was then coupled to diamino polyethylene glycol [M_W = 3400] in CH_2Cl_2 to give, after precipitation in ether, a white powder in 96% yield. The Z groups were removed by hydrogenolysis and the product, ([G2]-Lys-NH_2)$_2$-PEG dendron **5**, was isolated by precipitation in ether (99% yield). The structural identities of the various intermediates and dendritic macromolecules were determined by ^1H, ^{13}C NMR, and mass spectrometry (see Supporting Information).

A hydrogel starts to form within one minute of mixing aqueous solutions of the dendrimer or dendron with a poly(ethylene glycol) disuccinimidyl proprionate, **6**; PEG-NHS.[24] The hydrogels are optically transparent and Figure 1 shows the **5**:**6** hydrogel. All the hydrogels are formed using a 1:1 reactive group stoichiometry. The crosslinking reaction can be followed by infrared spectroscopy (IR), as shown in Figure 2a. For example, the IR stretch for the PEG-NHS at 1733 cm^{-1} disappears over time and the reaction between dendron **2** and **6** (1:1; 18 *w*/*w*%) is complete within 5000 seconds. As expected, the hydrogels swell when placed in an aqueous solution. Hydrogel swelling is linear with increasing concentrations of polymer

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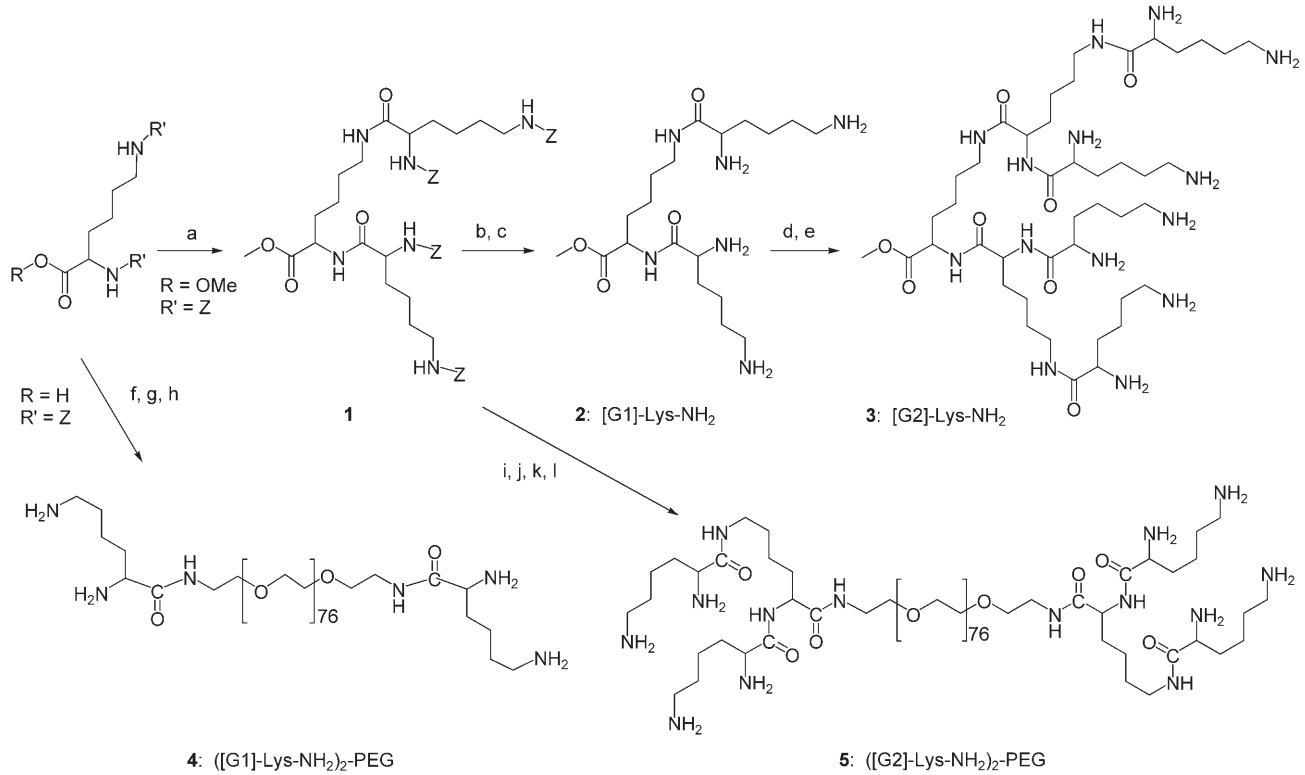

Scheme 1. Synthesis of dendrons and dendrimers. a) Z-Lys(Z)-OPFP, DMF, DIEA, HOBT, CH_2Cl_2, 25 °C for 24 h, 98% yield; b) H_2/Pd/C, MeOH, 6 h, 99% yield; c) 1 M HCl, 99% yield; d) Boc-Lys(Boc)-OPFP, DMF, CH_2Cl_2, DIEA, HOBT, 25 °C for 24 h, 70% yield; e) TFA 15% in CH_2Cl_2, 25 °C for 1 h, 99% yield; f) Z-Lys(Z)-OPFP, DMF, DIEA, HOBT, CH_2Cl_2, 25 °C for 24 h, 98% yield; g) PEG diamine $M_w = 3400$, HOBT, CH_2Cl_2, 24 h, 95% yield; h) H_2/Pd/C, MeOH, 6 h, 99% yield; i) 1 M NaOH/MeOH (50:50), 25 °C for 12 h, 85% yield; j) PFP, DCC, CH_2Cl_2, 25 °C for 24 h, 95% yield; k) PEG diamine $M_w = 3400$, CH_2Cl_2, HOBT, 24 h, 96% yield; l) H_2/Pd/C, MeOH, 6 h, 99% yield.

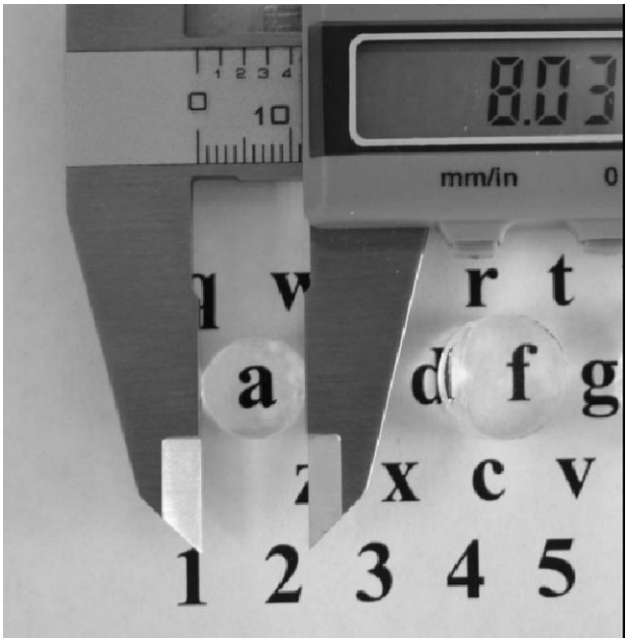

Figure 1. Photograph of **5**:**6** hydrogel before (a) and after swelling (f).

(Figure 2b). The extent of swelling ranges from approximately 150 to 800% depending on the dendron or dendrimer, and the concentration. The **4**:**6** and **5**:**6** hydrogels swell more than the **2**:**6** or the **3**:**6** hydrogels, consistent with the greater amount of PEG and overall hydrophilicity of hydrogels. Comparison of the hydrogels prepared from **2** or **3** with **6**, shows that the former swells slightly more, and the same trend is observed with the **4**:**6** and **5**:**6** hydrogels. This reflects the structure formed with the **2**:**6** and **4**:**6** hydrogels containing fewer crosslinks, at the same weight percent, when compared to the **3**:**6** and **5**:**6** hydrogels.

The rheological properties of the hydrogel before and after swelling were then investigated as a function of dendritic macromolecule and concentration. All the hydrogels show strong elastic properties with low Tang δ ($< 5°$). The values for the compressive (E) and complex moduli (G^*) increase logarithmically with increasing concentrations for all hydrogel compositions (Figure 3 and 4). The hydrogels formed between the dendrimer (**4** or **5**) and PEG activated ester (**6**) are described first. The hydrogels formed from **4**:**6** or **5**:**6** exhibit similar compressive moduli before swelling (Figure 3a) and range from approximately 50000 Pa to 250000 Pa with increasing concentration (not significantly different; p value = 0.041). After swelling, the moduli for the **4**:**6** and **5**:**6** hydrogels decrease. Going from the unswelled to swelled state, we observed a larger reduction (2X) in E for the **4**:**6** hydrogel in comparison to the **5**:**6** hydrogel at high weight percent. This result reflects the differences in swelling and network of the **4**:**6** hydrogel relative to the **5**:**6** hydrogel at high weight percent. The G^* for the **4**:**6** and **5**:**6**

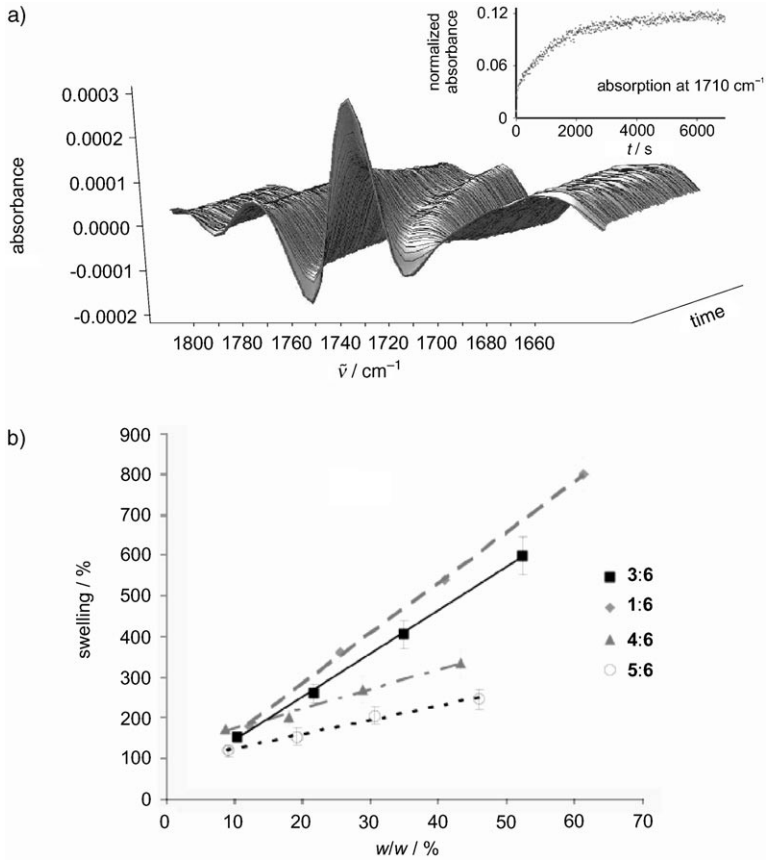

Figure 2. a) IR trace during crosslinking of the hydrogel. b) Swelling ratio vs. *w/w*.

hydrogels increases from approximately 5000 Pa to 20000 Pa with increasing concentration (Figure 3b). After swelling, the hydrogels exhibit a smaller *G**, and the values vary from 4000 Pa to 11000 Pa as a function of increasing weight percent.

The hydrogels prepared from dendrons **2** and **3** with the PEG activated ester (**6**) have different values for *E* and *G** compared with the hydrogels prepared with dendrimers **4** and **5**. Before swelling, the *E* increases from approximately 40000 to 300000 Pa for the **2**:**6** hydrogel and from approximately 20000 to 150000 Pa, for the **3**:**6** hydrogel with increasing weight percent (Figure 4a). At the same weight percent, hydrogels prepared from **2**:**6** have a larger *E* than those prepared from **3**:**6**. After swelling the *E* values decrease as expected, and the difference between the *E* values for before and after swelling, increases with increasing weight percent. The *G** values for the **2**:**6** hydrogel increase from approximately 4000 to 25000 Pa and for the **3**:**6** hydrogel from approximately 2000 to 14000 Pa. After swelling, all the hydrogels exhibit a smaller *G** with a decrease of about 50% for the **2**:**6** and 30% for **3**:**6** hydrogel. When comparing both systems, dendrons or dendrimers, before swelling the *E* and *G** of the hydrogels prepared from the dendrimers **4** and **5** are higher than the hydrogels prepared from the dendrons **2** and **3**. After swelling, the hydrogel prepared from dendron **2** shows a slightly higher *E* and *G** than with the other system. We selected the **2**:**6** hydrogel adhesive formulation for additional evaluation since it cured rapidly to form a hydrogel, possessed a *G** of 13000 Pa at 18 *w/w*%, and did not swell significantly. These characteristics afforded an adhesive which was soft and hydrophilic. The choice of this **2**:**6** hydrogel adhesive formulation over the other formulations was driven by several considerations including: 1) a minimal number of synthetic steps to obtain a functional material; 2) its low swelling ratio which should increase resident time on the site; 3) mechanical properties sufficient to secure the wound (previously reported results on in vivo corneal lacerations with photoactivated adhesive showed that a *G** greater than 10000 Pa was able to secure the wound);[19–22] and 4) a low viscous formulation that afforded good mixing to form a hydrogel.

To evaluate whether a hydrogel would close a common surgically made ophthalmic wound, we performed several in vitro vitrectomy experiments. A vitrectomy is the surgical removal of the clear, viscous proteinaceous fluid, called the vitreous, that fills the posterior two-thirds of the eye. A vitrectomy is performed to remove blood, debris, and scar tissue, that obscure the light path to the retina, resulting in blurred vision. The empty vitreous cavity is subsequently filled with a balanced salt solution or a bubble of gas. The vitreous is also removed if it is pulling on the retina causing retinal distortion or in some cases, retinal detachment. Once the vitreous is removed, retinal repair can be performed including removal of subretinal fluid, removal of epiretinal membranes, and repair of macular holes.

In order for surgeons to access the vitreous cavity and the retina, an incision is made in the sclera between the ciliary body and the retina, in an area called the pars plana. A diamond-shaped metal blade is typically employed, creating three separate, approximately 1.4 mm, linear sclerotomy wounds. One incision is required for the vitrector, an instrument that allows the vitreous to be aspirated into the cutting port where a tiny oscillating guillotine-like blade cuts the vitreous and removes it from the eye in a slow and controlled fashion. An infusion port is required to replace fluid in the eye and maintain proper intraocular eye pressure. A third port is required to insert a fiberoptic probe for intraocular illumination. Thus, at the end of each vitreo-retinal case, the surgeon is left with three sclerotomy wounds that must be repaired. Traditionally, these wounds have been closed using a suture such as vicryl.

Suture placement is time-consuming, traumatic to the tissue, and increases the risk of inflammation and vascularize-

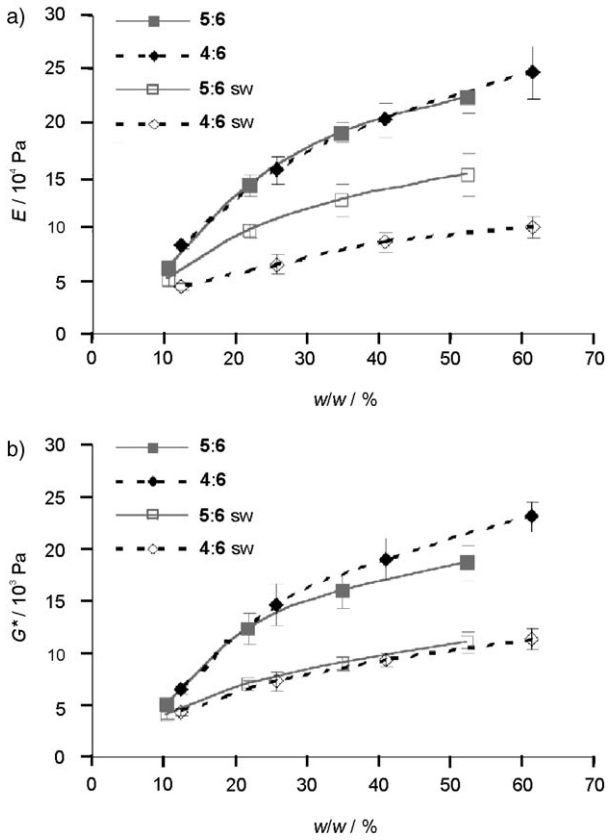

Figure 3. a) Compressive modulus (*E*) vs. *w*/*w* before and after swelling (sw) for the hydrogels **5**:**6** and **4**:**6**. b) Complex modulus (*G**) vs. *w*/*w* before and after swelling (sw) for the hydrogels **5**:**6** and **4**:**6**.

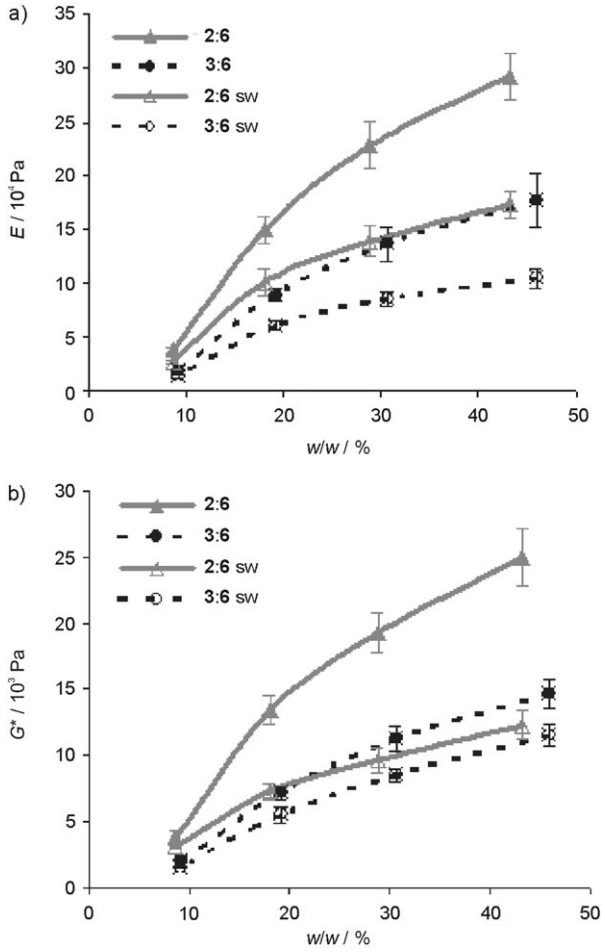

Figure 4. a) Compressive modulus (*E*) vs. *w*/*w* before and after swelling (sw) for the hydrogels **2**:**6** and **3**:**6**. b) Complex modulus (*G**) vs. *w*/*w* before and after swelling (sw) for the hydrogels **2**:**6** and **3**:**6**.

tion.[25–27] Ocular sealants are being evaluated as an alternative to sutures to improve the clinical outcome and reduce postoperative complications. The first sealants tested were superglues (or cyanoacrylates), as described by Webster and coworkers in the 1960s.[28] Cyanoacrylate adhesives have proven to be an effective therapeutic option in certain ophthalmic settings, such as sealing small corneal perforations.[28–34] However, the limitations of this system include application, effectiveness, toxicity, lack of biodegradation, and abrasiveness.[32,35–42] To determine if this hydrogel would effectively seal a 1.4 mm wound in the sclera, we performed a series of enucleated pig eye experiments to determine the pressure (that is the leaking pressure) at which intraocular fluid leaks from untreated, sutured, or hydrogel adhesive-repaired sclerotomy wounds.

In these preliminary experiments, we made one 1.4 mm full-thickness sclerotomy wound in the pars plana perpendicular to the limbus using a microvitreoretinal (MVR) blade in each of 17 porcine eyes. Seven of the wounds were left untreated and six wounds were closed using a traditional 3-pass running configuration with 7–0 vicryl suture. The remaining four wounds were sealed using the **2**:**6** formulation (Figure 5) which forms a soft and hydrophilic gel. An aqueous phosphate buffer solution of **2** and **6** was prepared such that the final total polymer *w*% of the combined solution was 18%. The solutions were combined and manually mixed for approximately 5-10 seconds using a 4.1 mm keratome blade. Next, an Opticel sponge was

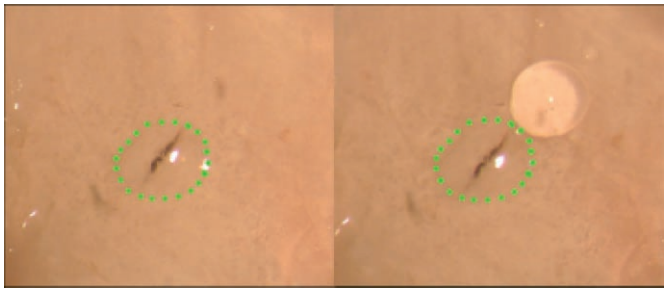

Figure 5. Left: photograph of a 1.4 mm full-thickness sclerotomy wound sealed with adhesive. Right: photograph of a wound immediately after failing to secure the wound at very high pressure (> 250 mmHg). Water droplet on upper right of the adhesive is seen. The area where the adhesive is present is highlighted in green.

used to dry the wound. The **2**:**6** solution was placed on the bottom of a keratome blade (5–10 μL) and then applied to cover the wound. A hydrogel patch formed within 30 seconds. We then infused balanced salt solution into the vitreous cavity at a rate of 8 mL h^{-1} and the infusion was continued until the wound leaked, at which point the leaking pressure was noted. If the wound had not leaked before a pressure greater than

250 mmHg, the experiment was terminated and the leaking pressure recorded as 250 mmHg. The untreated wounds leaked at an average pressure of 6 ± 3 mmHg. The sutured wounds leaked at an average pressure of 140 ± 68 mmHg. The wounds treated with the biodendritic adhesive all sustained pressures above 250 mmHg without leaking. Since normal intraocular pressure is about 15 mmHg, the sealant possesses sufficient strength to secure the wound.

To gain further insight into the possible modes of adhesion, we determined whether covalent crosslinking between the protein amines and the hydrogel components was responsible for the adhesion strength. We performed a similar sealing and leaking experiment as described above with a 1.4 mm full-thickness sclerotomy wound in two additional eyes. The wound was first treated with a pH buffer of 9 to activate the amines and then the wound was exposed to a solution of PEG-NHS, **6**, which reacts with the protein amines. Upon increasing the pressure, the wound leaked at low pressure (<10 mm Hg) confirming that treatment with **6** alone does not seal the wound. Next, we treated the wound with phosphate buffer at pH 9 to hydrolyze any remaining activated ester and then sealed the wound with the **2**:**6** dendritic adhesive. The wound had not leaked up to a pressure of 250 mmHg. If the adhesion was primarily a result of covalent crosslinking with the surface proteins, then the adhesive should not have been able to secure wound. This result suggests that covalent crosslinking to the protein amines is not the primary means of adhesion to the tissue, and that an interpenetrating network with the scleral tissue is more likely responsible for the adhesion.[16]

In summary, lysine based dendrons and lysine-PEG-based dendritic macromolecules were prepared and crosslinked by acylation of the amines with the PEG-activated ester. The crosslinking reaction occurs quickly at room temperature and at neutral pH to afford an optically clear hydrogel adhesive. The sclerotomy wounds treated with the dendritic hydrogel adhesive sustained higher leaking pressures than traditionally sutured wounds. The ease of application and the resulting performance of these hydrogels indicate that these biomaterials may be a suitable alternative to sutures for closing sclerotomy wounds, as well as for other ocular wounds. Synthetic approaches to hydrogels that use precursor macromolecules of well-defined composition and structure provide significant research opportunities. With regards to new soft materials for the medical and biotechnology industries, these results provide further impetus to design, synthesize, characterize, and optimize tailored-materials through specific alterations at the molecular level.

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Keywords: dendrimers · hydrogels · materials · ophthalmology · polymers

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