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Dendritic Macromers as in Situ Polymerizing Biomaterials for Securing Cataract Incisions

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Dendritic macromolecules are versatile materials for medical applications since the chemical composition, structure, and molecular weight can be precisely controlled.[1−7] Moreover, the hyperbranched globular structure permits diverse and extensive surface functionalization, which facilitates subsequent intermolecular cross-linking, surface adhesion, or biological recognition. Cross-linkable dendritic macromolecules are known to form macroscopic gels, including hydrogels.[8] Hydrogels are of general interest and are used in applications ranging from drug delivery to wound healing.[9,10] We are exploring the latter application using dendritic polymers that are derived from monomers known to be natural metabolites or biocompatible.[8,11−13] The advantages of dendritic macromers for hydrogel formation include high cross-linking densities at low polymer concentration, varied physical properties through judicious choice of the macromer structure, and low viscous aqueous solutions for injection in an in vivo site of irregular shape for subsequent cross-linking to form a well-integrated polymer network. Recently, we have successfully repaired full-thickness 4.1-mm corneal lacerations in enucleated eyes and chicken eyes in vivo using a hydrogel sealant produced from photo-cross-linkable poly(glycerol-succinic acid)−poly(ethylene glycol) hybrid dendritic−linear macromolecules.[13,14] We are currently investigating alternative cross-linking chemistries to light initiation that spontaneously afford a hydrogel within a few minutes at room temperature and at neutral pH. Herein, we report the synthesis of peptide dendrons, the formation of cross-linked hydrogels, the analysis of hydrogel physical properties, and the use of these biomaterials to seal a corneal incision: the wound created during a typical cataract procedure.

To afford a hydrogel under mild aqueous conditions while maintaining chemoselective cross-linking and a high tolerance to a range of chemical functionalities, we selected the formation of a thiazolidine linkage between the macromers to render a cross-linked hydrogel (Figure 1). This linkage between an aldehyde and a cysteine 1,2 aminothiol group occurs quickly at room temperature and is stable from pH ≈ 3−9. Moreover, this coupling reaction belongs to a family of peptide ligation reactions used to prepare large proteins and enzymes in aqueous solution that were otherwise unattainable using solid-phase peptide synthetic methods.[15−17] To use such a strategy for hydrogel formation, lysine-based peptide dendrons[18,19] possessing either four or eight *N*-terminal cysteine residues were synthesized (Scheme 1).

The pentafluoro-ester strategy was used to synthesize the dendrons since it afforded higher amide coupling reaction yields than coupling reactions employing BOP, DCC, EDCI, or oxalyl chloride. First, the pentafluoroesters of ZLys(Z)OH and IsoCys(Boc)OH were prepared using DCC and 2,3,4,5,6-pentafluorophenol (PFP). Next, ZLys(Z)OPFP was coupled to LysOMe·2HCl in the presence of DIEA and HOBT. The Z group was removed via catalytic hydrogenolysis (Pd/C (10% w/w) and H_2). The dendron **3** was prepared by coupling IsoCys(Boc)OPFP to LysLys(Lys)-OMe·4HCl followed by removal of the Boc and iso-protecting groups of cysteine using TFA and 1 N HCl in MeOH, respectively.

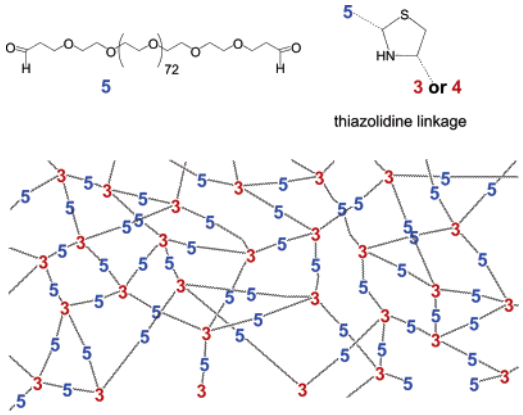
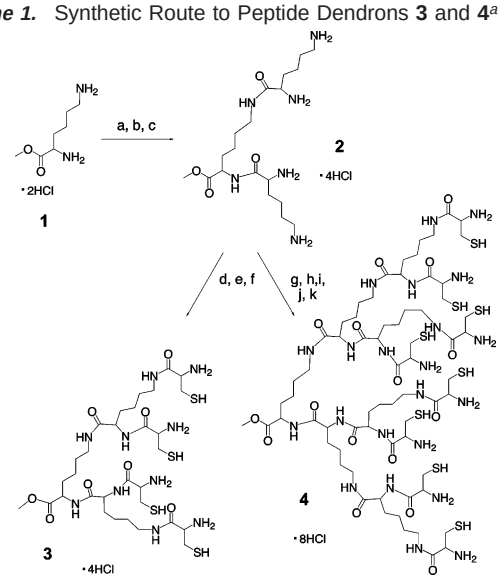


Figure 1. Structure of macromer **5** and the thiazolidine linkage formed by the reaction of **3** or **4** and **5**. Schematic of an idealized cross-linked hydrogel formed between **3** and **5**.

Scheme 1. Synthetic Route to Peptide Dendrons **3** and **4**[a]



[a] Reactants and conditions: (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) HCl 1 N, 25 °C for 5 min, 99% yield; (d) iso-Cys(Boc)-OPFP, DMF, CH_2Cl_2, DIEA, HOBT, 25 °C for 24 h, 96% yield; (e) TFA 17% in CH_2Cl_2, 25 °C for 2 h, 97% yield; (f) HCl 1 N/MeOH 50/50, 25 °C for 4 h, 92% yield; (g) Boc-Lys(Boc)-OPFP, DMF, CH_2Cl_2, DIEA, HOBT, 25 °C for 24 h, 65% yield; (h) TFA 15% in CH_2Cl_2, 25 °C for 1 h, 99% yield; (i) iso-Cys(Boc)-OPFP, DMF, CH_2Cl_2, DIEA, HOBT, 25 °C for 24 h, 96% yield; (j) TFA 17% in CH_2Cl_2, 25 °C for 2 h, 97% yield; (k) HCl 1 N/MeOH 50/50, 25 °C for 4 h, 99% yield.

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Figure 2. (left) Photograph of a synthesized **3·5** hydrogel and (right) photograph of a **3·5** hydrogel sealant-repaired 3-mm clear corneal incision (incision is between the two purple dots and gel is within the blue border).

The larger dendron **4** was prepared in a similar manner except that an additional Lys coupling reaction was performed. In this reaction, the BocLys(Boc)OH was used instead of ZLys(Z)OH to increase the solubility of the larger Lys-dendron intermediate in organic solvents. Finally, the Boc and iso-protecting groups of cysteine were removed using TFA and 1 N HCl in MeOH, respectively, to afford dendron **4**. The dendrons were fully characterized by NMR, FAB-MS, and elemental analysis (see Supporting Information).

To prepare the hydrogels, dendron **3** or **4** was reacted with poly(ethylene glycol dialdehyde) of 3400 M_w (**5**, PEG-DA) in HEPES buffer at pH = 7.4 (Figure 1). The ratio of cysteine to aldehyde was 1:1, and the total concentration of polymer in solution was either 30 or 50% w/w. A hydophilic gel formed spontaneously within three minutes upon mixing the two aqueous solutions at either concentration (Figures 1 and 2). The gels exhibited viscoelastic properties. Cylindrical hydrogel samples of 8-mm diameter and 2-mm thickness were prepared and analyzed after setting at 25 °C for 24 h. The mechanical properties were measured at a frequency of 1 Hz. The complex modulus (G*) for the 30% w/w hydrogels prepared from **3** or **4** and **5** was 3.8×10^3 and 1.0×10^4, respectively. The increase in modulus is consistent with the increase in cross-linking density present in the **4·5** hydrogel.[20] The hydrogels were transparent, and the **3·5** hydrogel had a refractive index of 1.397 at 37 °C, similar to the human cornea ($n^{37} = 1.376$).

Cataract removal is the most commonly performed ophthalmic surgical procedure, with 11 million/year performed worldwide, and this number is expected to increase with the aging demographics. A cataract is the clouding of the lens that decreases vision. Today, surgeons break up and remove the cataract using ultrasound energy and implant a synthetic intraocular lens, all through an incision in the cornea. At the conclusion of the procedure, this corneal incision is either left alone to "self-seal" or closed with nylon sutures.

Suturing inflicts trauma to the tissue and has risks such as increasing inflammation and vascularization.[21−23] Moreover, sutures need to be removed post-operatively. The "self-seal" approach also has its drawbacks, including leakage and increased risks of infection (i.e., endophthalmitis).[24] To determine whether this hydrogel sealant would secure a corneal incision, we performed a series of experiments on enucleated eyes to evaluate the leaking pressures of self-sealed, suture, or hydrogel sealant-repaired incisions.

A 3-mm clear corneal linear incision was made in an enucleated eye. This wound was either left to self-seal or closed using one interrupted 10-0 nylon suture or the hydrogel sealant. For the hydrogel sealant, dendron **3** and PEG-DA, **5**, were mixed quickly at room temperature, and then ~5 μL of the hydrogel sealant was applied to the incision (Figure 2). Within 5 min of repairing the incision, saline was injected in the anterior chamber via a syringe pump until the repaired incision leaked. In this in vitro study, the mean leaking pressure for the self-seal ($N = 7$) and suture ($N = 2$)-treated eyes were 24 ± 8 and 54 ± 16 mmHg, respectively. The leaking pressure for the eyes repaired with hydrogel sealant ($N = 8$) was 184 ± 79 mmHg. Normal intraocular pressure is between 12 and 16 mmHg. The incision is not sealed using only the dendron or PEG-DA hydrogel precursors. The hydrogel sealant secures the clear corneal incision and withstands higher pressures and stress placed on a wound than conventional suture or self-sealed treated wounds. The procedure with the hydrogel sealant is facile and requires less surgical time than conventional suturing (4−6 times) and does not inflict additional tissue trauma. The cross-linked hydrogel sealant is transparent, adhesive, elastic, hydrophilic, and acts as a physical barrier to the ocular surface.

In summary, a new in situ polymerizing hydrogel is reported that gels within a few minutes from the multiple thiazolidine linkages formed between the cysteine residues of the dendron and the poly(ethylene glycol) macromers. The use of a hydrogel sealant as opposed to nylon sutures or the self-seal approach in cataract surgeries may provide a facile method to safely and effectively seal the incision while reducing potential complications. These results further support the synthesis and evaluation of dendritic macromolecules for medical applications, where a high level of molecular control can be used to vary and optimize chemical, physical, and mechanical properties.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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