Published in final edited form as:

Biomacromolecules. 2011 May 9; 12(5): . doi:10.1021/bm200039s.

Synthesis and Characterization of Dendron Cross-linked PEG Hydrogels as Corneal Adhesives

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Abstract

In pursuit of a wound-specific corneal adhesive, hydrogels formed by the reaction of propionaldehyde, butyraldehyde, or 2-oxoethyl succinate-functionalized poly(ethylene glycol) (PEG) with a peptide-based dendritic cross-linker (Lys3Cys4) were characterized. These macromers react within minutes of mixing to form transparent and elastic hydrogels with *in vitro* degradation times that range from hours to months based on the type of bonds formed during the cross-linking reaction – either thiazolidine or pseudoproline. The mechanical properties of these materials, determined *via* parallel plate rheology, were dependent upon the polymer concentration, as was the hydrogel adhesive strength, which was determined by lap shear adhesive testing. In addition, these hydrogels were efficacious in closing *ex vivo* 4.1 mm central corneal lacerations: wounds closed with these hydrogel adhesives were able to withstand intraocular pressure values equivalent to, or in excess of, those obtained by closing the wounds with suturing.

Keywords

Dendron; dendrimer; hydrogel; peptide ligation; adhesive; sealant; cornea; corneal wounds

Introduction

Each year, more than 15 million individuals worldwide require treatment for the repair of ocular wounds caused by trauma or disease, as well as incisions for cataract, LASIK, corneal transplant, and other ophthalmic surgeries.¹⁻³ Ocular tissue is difficult to repair because its structure and curvature play an integral part in the eye's overall function; thus, improper juxtaposition of the wound edges or shear force on the wound surface during healing can cause astigmatism. Additionally, vascularization of the cornea due to inflammation can decrease the transparency of the tissue and reduce the patient's visual acuity.

In the most common clinical practice, surgeons use thin-filament nylon sutures to close ocular wounds, and depending on the type and size of the wound, multiple sutures are often required. Unfortunately, sutures do not reduce the amount of time required for the surgical procedure or the invasiveness of the surgical procedure. Furthermore, sutures do not actively participate in healing. It is no surprise, then, that the use of sutures to close ocular wounds inflicts additional trauma to the tissue, provides a path for microbial contamination, and incites inflammation, vascularization and uneven healing.⁴⁻⁶ Sutures may also become loose

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COI: MWG is a Co-founder of Hyperbranch Medical Technology, which has developed and commercialized an ocular hydrogel sealant for the closure of corneal wounds.

or even be broken postoperatively. These inherent risks have spurred researchers to develop adhesive materials designed to improve the current standard of care in ophthalmology.³

Research into improved adhesives for ophthalmic or other medical applications has largely focused on the development of hydrogels that can be applied *in situ* to seal the wounds by cross-linking around microscopic features of the tissue surface and/or by bonding directly to the tissue.^{3,7} Hydrogel adhesives have been produced from natural polymers such as hyaluronic acid, chondroitin sulfate, and collagen.⁸⁻¹⁹ These polymers are cross-linked *via* reaction of synthetically-introduced functional groups (such as methacrylate, adipic dihydrazide, or aldehyde) or by enzymatic methods. Although these adhesives are efficacious in closing a variety of wounds, including examples in ophthalmology,^{12,15,20} there is interest in investigating synthetic alternatives, which may offer additional advantages in terms of cost, purity, manufacturing, and performance. Hydrogel adhesives composed of synthetic polymers (many of which are poly(ethylene glycol) (PEG)-based) have been cross-linked by photo-activated free radical processes or by nucleophilic substitution reactions.^{6,21-31} These materials are promising candidates for ophthalmic adhesives due to their biocompatibility, tunable mechanical properties, and degradation time that can be tailored, potentially, to match the time scale of wound healing.

Previous research by Grinstaff *et al.* has included the development of hydrogel adhesives based on dendritic polymers³²⁻³⁵ for the repair of *ex vivo* and *in vivo* ocular wounds such as central corneal lacerations,^{6,22,23} clear corneal cataract incisions,^{26,30} flaps from LASIK surgery,²⁵ scleral incisions,³⁶ and 8 mm penetrating keratoplasties.^{24,31} Adhesive hydrogels for these applications are cross-linked upon irradiation with 514 nm laser light in the presence of a photo-initiator or by nucleophilic substitution reactions that occur under physiological conditions of pH and temperature, as highlighted elsewhere.³⁷ In particular, hydrogels produced from the reaction of a peptide-based dendron with aldehyde-functionalized PEG have shown efficacy for the *ex vivo* repair of cataract surgery incisions^{26,30} and corneal autografts.³¹ In this paper, we further explore the properties of hydrogels prepared from the reaction of a peptide dendron (Lys₃Cys₄) with three different types of homobifunctional aldehyde-terminated PEG macromers: PEG-propionaldehyde (P-Ald PEG), PEG-butyraldehyde (B-Ald PEG), and PEG-2-oxoethyl succinate (E-Ald PEG). The thiol and amine functionalities of the cysteine residues at the terminus of the Lys₃Cys₄ dendron react upon mixing with the P-Ald or B-Ald PEG to form thiazolidine linkages, which are susceptible to hydrolysis. In a similar manner, the reaction between the E-Ald PEG and the Lys₃Cys₄ dendron yields thiazolidine linkages; however, these structures subsequently rearrange *via* O,N-acyl migration to form the more stable pseudoproline linkages as depicted in Figure 1(f). The effect of hydrogel formulation and cross-link type on material degradation and physical properties as well as *in vitro* cytotoxicity and efficacy in repairing *ex vivo* full thickness 4.1 mm central corneal lacerations, are reported herein.

Materials and Methods

General

The following abbreviations are used throughout the text: B-Ald PEG = PEG-butyraldehyde; CBS = calf bovine serum; DMEM = Dulbecco's modified Eagle's medium; DMSO = dimethylsulfoxide; DPBS = Dulbecco's phosphate-buffered saline; E-Ald PEG = PEG-2-oxoethyl succinate; EDTA = ethylenediaminetetraacetic acid; EMEM = Eagle's minimal essential medium with Earle's balanced salt solution; FBS = fetal bovine serum; MTS = 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; P-Ald PEG = PEG-propionaldehyde; PEG = poly(ethylene glycol). All PEG used had a molecular weight of 3,400 g/mol.

Statistical evaluation

A two tails t-test was used and results were considered statistically different for $p < 0.05$.

Reagents

All chemicals for synthesis and hydrogel formation were purchased from Sigma Aldrich or Acros Organics and used as received, unless otherwise noted.

Hydrogel Formation

The Lys₃Cys₄ dendron, E-Ald PEG, and P-Ald PEG were synthesized as previously described.^{30,31} B-Ald PEG was purchased from Laysan Bio. The hydrogels characterized herein were produced by reaction of the Lys₃Cys₄ dendron with each of the three PEG polymers in a stoichiometric ratio of one thiol to one aldehyde, at the desired overall concentration (Lys₃Cys₄ + PEG) ranging from 10 – 50 % by weight. In the remainder of the text, the following notation will be used to describe the hydrogel adhesives: P-Ald hydrogel (composed of Lys₃Cys₄ dendron and P-Ald PEG), B-Ald hydrogel (composed of Lys₃Cys₄ dendron and B-Ald PEG), and E-Ald hydrogel (composed of Lys₃Cys₄ dendron and E-Ald PEG). Hydrogel samples for characterization studies were formed by mixing the Lys₃Cys₄ (dissolved in buffer at pH 9) with the desired PEG polymer (dissolved in buffer at pH 7.4) and allowing the solution to cross-link in cylindrical Teflon molds (8 mm diameter, 4 mm height). Although gelation was apparent within 3 minutes of mixing the components, the molds were placed in a sealed container with high humidity for 24 hours for the B-Ald, and P-Ald hydrogels and 10 min for the E-Ald hydrogel to ensure cross-linking and hydrogel formation. The resulting hydrogels were carefully removed from the molds and placed in buffer until swelling reached equilibrium (i.e. no further change in swelled sample mass). The P-Ald hydrogels had lost their structure after approximately 3 hours of swelling due to degradation.

Hydrogel Characterization

The mass of each sample was recorded immediately after cross-linking (the as-prepared state or relaxed state, denoted with the subscript “R”), and again after samples had swelled to equilibrium (the swelled state, denoted with the subscript “S”). The hydrogels were stored in buffer solution and weighed daily until their compressive and shear moduli reached the lower measurement limit of the rheometer ($E < 1\text{ kPa}$, G' and $G'' < 1\text{ Pa}$, using test methods described below), at which point the degradation time was noted. The refractive index and percent transmission of visible light through hydrogel samples in the relaxed and swollen states were determined using a refractometer (Rudolph J257) and UV-vis spectrophotometer (HP 8453), respectively at 25 °C.

The mechanical properties of hydrogels were measured by analysis of sample strain in response to unconfined compression and shear forces using a parallel plate rheometer (AR1000 Rheometer, TA Instruments) as described previously.³⁸ Briefly, an initial normal force of 0.2 N was applied to the hydrogel, followed by compression to a total strain of 10%. After a brief relaxation period, the hydrogel samples were subjected to shear strains of 0.4%. The resulting stress-strain curves were subjected to linear regression in order to obtain values for the compressive modulus and the shear modulus respectively.

Cytotoxicity Testing

The effect of the hydrogel components (Lys₃Cys₄ dendron, B-Ald PEG, and E-Ald PEG) as well as the preformed, intact hydrogels (B-Ald hydrogel and E-Ald hydrogel) on the *in vitro* viability of rabbit corneal fibroblasts and NIH-3T3 fibroblasts was measured. Rabbit corneal fibroblasts were obtained from ATCC (SIRC CCL-60) and cultured in EMEM (ATCC)

supplemented with 10% FBS (Fisher Scientific) at 37 °C in an atmosphere containing 5% CO₂. The culture media was changed every 3 days and subculture was conducted every 5 days by enzymatic dissociation with 0.25% trypsin/EDTA solution (ATCC) in DPBS. NIH-3T3 cells were obtained from ATCC and cultured in DMEM containing 10% CBS; these fibroblasts were subcultured every 3 days by enzymatic dissociation with 0.25% trypsin/EDTA solution (ATCC) in DPBS.

In order to determine the effect of the Lys₃Cys₄ dendron, E-Ald PEG, and B-Ald PEG on cellular viability, cells were plated in 96-well plates at a seeding density of 5000 cells/well and then left to attach for 18 hours. The cells were then exposed to solutions containing the hydrogel components in media at 0.05, 0.5 and 5 wt% concentrations for 3 hours. Cell viability was measured using a standard tetrazolium salt viability assay (MTS assay, Invitrogen). The MTS reagent was used in a 1:5 dilution (MTS: media) with an incubation time of 1 hour: after incubation with MTS, the absorbance values of solution aliquots at 592 nm were measured. Cell viability was determined by comparing absorbance values for solution aliquots from wells with cells exposed to hydrogels to those from control wells with cells that were not exposed. In addition, the cytotoxicity of the intact hydrogels was evaluated by seeding cells at a density of 20,000 cells/well in 24-well plates. After allowing 18 hours for cell attachment, hydrogels were formed on transwell membrane inserts (Corning) and cultured above the cells in full contact with the media for 24 hours (hydrogel volume = 5 µL). Finally, the MTS assay was conducted as described above.

***In Vitro* Adhesive Performance**

The adhesive strength of the E-Ald and B-Ald hydrogels was determined by lap shear adhesive testing. Fresh rabbit eyes were obtained from Pel-Freez Biologicals (Rogers, AR) and shipped in DMEM containing gentamicin, penicillin, streptomycin, and amphotericin B. All processing and dissection were conducted within 24 hours of the time of death. The central cornea of each eye was removed with an 8 mm circular trephine blade and rinsed with sterile DPBS; a rectangular strip (8 mm by 4 mm) was then cut from each cornea. For each adhesion test, two strips of corneal tissue were gently blotted with a sponge to remove excess fluid and then adhered with a small amount of hydrogel adhesive (5 µL). After waiting approximately 5 minutes for cross-linking to occur, the end of each strip was then placed in standard test grips of a materials testing device (Instron 5848 Microtester), and as the grips were pulled apart at a crosshead speed of 0.5 mm/s, the force required to cause adhesive failure was measured.

Subsequently, each type of hydrogel was used to close an *in vitro* 4.1 mm central corneal laceration in an enucleated human eye, as previously described.²² In brief, corneas were removed from the eyes and clamped onto a testing chamber that was connected to a pressure transducer as well as to a syringe pump filled with saline solution. Next, a keratome blade was used to induce a full-thickness, 4.1 mm incision in the cornea. The wound edges were carefully aligned and gently blotted with a sponge to remove excess fluid. The hydrogel components were reconstituted in buffer, mixed, and applied to the wound surface (hydrogel volume = 5 µL). After waiting 5 minutes for cross-linking to occur, the chamber was slowly pumped with saline (8 mL/hour) until leakage of saline from the wound site was observed under a surgical microscope. The maximum pressure obtained during each test was recorded. The same test protocol was also conducted after closing the incision with a single suture.

Results and Discussion

The purpose of this study was to explore two different cross-linking chemistries used to prepare hydrogels³⁹⁻⁴⁷ *via* either pseudoproline or thiazolidine linkages. These hydrogels

were produced by the reaction of each of three different aldehyde functionalized PEG polymers with the peptide-based Lys₃Cys₄ dendron, as shown in Figure 1. This peptide ligation approach was chosen for both chemical and clinical reasons. From a chemistry perspective, peptide-ligation reactions have been used successfully to prepare proteins and enzymes *via* the coupling of large peptides in aqueous solution that were otherwise unattainable using solid-phase peptide synthesis.⁴⁸⁻⁵⁵ Importantly, these coupling reactions can be done with unprotected amino acids - thus demonstrating the tolerance of this reaction to other chemical functionalities.⁴⁸⁻⁵⁵ Since its first report, this approach has been used in many areas including the preparation of glycoproteins⁵⁶ and protein-polymer conjugates.⁵⁷

We selected this cross-linking chemistry over the more typical reactions reported in the literature such as Michael additions (PEG diacrylates or PEG vinyl sulfones and PEG diamines or dithiols), PEG-(N-hydroxy succinimide) (NHS) (or analogous imidazole or nitrobenzyl active esters with PEG diamines or dithiols), and Schiff base reactions (PEG diamines and PEG dialdehydes) for several reasons. First, the peptide ligation reactions can be performed at mild conditions like the Schiff base reactions whereas the NHS and Michael addition systems typically require basic conditions for rapid (< 1 minute) reactions. Second, no chemical byproducts are generated by the peptide ligation reaction; it is a self-contained reaction like the Michael addition and Schiff base reactions (NHS, imidazole, nitrobenzyl active esters afford byproducts). Third, the choice of substrate for the peptide ligation reaction dictates whether the product is stable or reversible, thus allowing for additional control over material degradation rate for the desired application. Tam et al have reported on the stability difference between the thiazolidine and pseudoproline linkage, where the pseudoproline linkage is significantly more stable than the thiazolidine.⁵¹ Finally, this peptide ligation strategy has a high tolerance to a range of other chemical functionalities as documented in its use in the protein synthesis field. This peptide ligation approach has some versatility, as Messersmith *et al.* has recently used a similar strategy to prepare crosslinked hydrogels in which a free thiol was generated for subsequent chemical coupling to an RGD peptide to mediate cell adhesion throughout the hydrogel.⁵⁸ The above rationale and observations strongly support the use of these reactions or other similar peptide ligations reactions for the synthesis of hydrogels.

From a clinical viewpoint, ease of use, safety, and efficacy are critical metrics for the adoption of a hydrogel based ocular adhesive.³ Our previous studies using a photo-activated adhesive provided us with the material and surgical procedural design requirements as well as a hydrogel formulation for successfully repairing *ex vivo* and *in vivo* corneal lacerations.^{6,22,23} However, we have learned from subsequent discussions with clinicians that a photo-activated system in ophthalmology is less than ideal due to the documented argon ion laser damage to retinal and corneal tissue caused by incorrect laser use,⁵⁹⁻⁶⁷ heat generated upon crosslinking with a laser (2 - 3 °C), and the additional procedural complications of requiring a laser. Thus, adhesive systems that can be easily mixed, applied to the wound, and crosslinked *in situ* to form a hydrogel are favored and may garner faster acceptance by physicians.

Specifically, a tri-lysine dendron with cysteine residues located at its termini (Lys₃Cys₄) was reacted with each of three different types of homobifunctional aldehyde-terminated PEG macromers: PEG-propionaldehyde (P-Ald PEG), PEG-butyraldehyde (B-Ald PEG), or PEG-2-oxoethyl succinate (E-Ald PEG) under mild conditions of pH and temperature to form hydrogels. The reaction between the thiol and amine functionalities of the cysteine residues at the terminus of the Lys₃Cys₄ dendron and the aldehyde functionalities of either P-Ald PEG or B-Ald PEG affords thiazolidine linkages (Figure 1 (e)); whereas the reaction of the Lys₃Cys₄ with E-Ald PEG results in pseudoproline linkages after thiazolidine formation (Figure 1(f)). The reaction was followed by NMR spectroscopy using a model

system consisting of a cysteine methyl ester and a tetra-ethylene glycol diester-aldehyde.^{30 68} The appearance of the new α methylene ester proton resonance at 3.88 ppm for the thiazolidine intermediate and the disappearance of the starting α methylene aldehyde proton resonance at 4.05 ppm confirmed the first step of the reaction. Next, the *O,N* acyl rearrangement was observed as the α methylene ester peak at 3.88 ppm disappeared and a new peak corresponding to the α methylene alcohol at 3.80 ppm appeared, thus indicating the final pseudoproline product. The reaction has a $t_{1/2}$ of 10 hours. Upon mixing the macromers at 10, 20, 30 or 50 wt%, all three reactions gave transparent, intact hydrogels within 3 minutes (Figure 2).

The optical properties of the Lys₃Cys₄ cross-linked hydrogels, in comparison to those of the human cornea, are shown in Table 1. The hydrogels possess a lower refractive index than the human cornea. In the as-prepared or relaxed state (i.e., before swelling), the refractive index and transmission of light through hydrogel samples varies from 1.34 – 1.37 and 86 – 91%, respectively for the hydrogel samples (10-50 wt%). After swelling, however, all hydrogel samples exhibit optical properties that are roughly similar, with refractive index and transmission values of 1.34 and 91%, respectively. Ideally, an ophthalmic adhesive should transmit as much light as possible with a low refractive index so as to reduce visual aberrations.

The P-Ald, B-Ald, and E-Ald hydrogels possess degradation times of 3 hours, 1.5 weeks, and 6 months, respectively. The degradation times were similar regardless of the concentration used. Due to the reversible nature of thiazolidine cross-links formed in the production of P-Ald and B-Ald hydrogels,⁶⁹ these materials degrade more quickly than E-Ald hydrogels. The tunable degradation time of these hydrogel adhesives will be useful for repairing wounds that have healing times ranging from days (e.g., small corneal lacerations) to months (e.g., corneal transplants). The swelling characteristics of the B-Ald and E-Ald PEG hydrogels are shown in Figure 3. The P-Ald hydrogels degrade during equilibrium swelling and, as a result, only properties for these hydrogels in the relaxed state (before swelling) could be reported. A representative image of the swelled E-Ald hydrogels is shown in Figure 2. The swelling ratio, *Q* (defined here as the ratio between hydrogel sample weights in the swollen and relaxed states), increases with increasing initial polymer content (percent by weight) for both B-Ald and E-Ald hydrogels. The more PEG macromer in the sample, the more sites for hydration are present and thus more water is absorbed during the equilibrium swelling process. The effect of this swelling is shown in Figure 3b, which relates the polymer volume fraction of hydrogels in the swollen ($v_{2,S}$) and relaxed ($v_{2,R}$) states. These data demonstrate that the polymer volume fraction of the swollen hydrogels cannot be increased to more than ~ 0.1 , regardless of the polymer volume fraction in the relaxed state, most likely because the hydrogel network mesh size is essentially fixed by the size of the constituent macromers.

The relationship between hydrogel mechanical properties and initial polymer content is shown in Figure 4 for samples in the relaxed and swollen states. Overall, the hydrogel formulation can be tuned to achieve compressive modulus values ranging from 20 to 1000 kPa and shear modulus values ranging from 1 to 200 kPa before swelling with initial polymer contents of 10 to 50% (by weight). As expected, the magnitude of the hydrogel compressive and shear modulus values is dependent upon the polymer concentration with increased polymer content affording greater mechanical properties. The compressive modulus and shear modulus values undergo a dramatic decrease upon swelling to equilibrium, with both the B-Ald and E-Ald hydrogels exhibiting similar behavior. After swelling, the hydrogels possess compressive modulus values of 1 to 160 kPa and shear modulus values of 1 to 15 kPa. The compressive and shear moduli of the swollen hydrogels are dependent on polymer content, but to a significantly lesser extent than hydrogels in the

relaxed state. The slight increase of E and G^* in the swollen state reflects the increase in mass and cross-links per volume in the higher wt% samples.

The cytotoxicity of the constituent macromers was evaluated by exposing media with 0.05, 0.5 and 5 % (by weight) concentrations of dendron, B-Ald PEG, and E-Ald PEG to cells for 3 hours, as shown in Figure 5. Rabbit corneal fibroblasts were employed in this *in vitro* study in order to model the response of corneal cells to the adhesive. Additional testing was conducted using NIH-3T3 fibroblasts, as this cell line is widely used for cytotoxicity studies in the literature and is required for cytotoxicity studies as described in the FDA G95-1 guidelines. Exposure to the Lys₃Cys₄ dendron resulted in only minimal loss of viability to rabbit corneal fibroblasts (Figure 5a) and NIH-3T3 fibroblasts (Figure 5b) over this concentration range. The E-Ald PEG and B-Ald PEG also exhibited minimal cytotoxicity at low concentrations, but caused a substantial reduction in cell viability of both the rabbit corneal fibroblasts (Figure 5a) and NIH-3T3 fibroblasts (Figure 5b) at the highest concentration tested.

In addition, cytotoxicity of preformed hydrogels to rabbit corneal fibroblasts and NIH-3T3 fibroblasts was also evaluated. The viability of each cell line after exposure to the hydrogel samples at initial polymer concentrations of 10, 20, and 50 wt% for 24 hours is shown in Figure 5. The effect of E-Ald and B-Ald hydrogels on rabbit corneal fibroblast viability was generally negligible (Figure 5c), although exposure of these cells to the 50 wt% E-Ald hydrogel caused a slight reduction in viability. The NIH-3T3 cell line was more sensitive to hydrogel exposure with a decrease of 20 - 30% in viability compared to the untreated control samples (Figure 5d).

Next, the *in vitro* performance of the B-Ald and E-Ald hydrogels was evaluated by measuring the materials' adhesive strength and efficacy in repairing 4.1 mm central corneal lacerations. The tensile stress required to cause adhesive failure of corneal tissue joined with the hydrogel adhesive, shown in Figure 6a, was dependent upon polymer content for both the E-Ald and B-Ald hydrogels. To confirm that both of the adhesive components are needed, we performed additional tests by applying each individual macromer (Lys₃Cys₄, B-Ald, E-Ald) to tissue sections at the same concentration as used above. In all cases no adhesion of the tissue was observed using the Lys₃Cys₄ dendron or the B-Ald PEG or E-Ald PEG alone, confirming that application of both the PEG aldehyde and Lys₃Cys₄ dendron is required to form the tissue adhesive. Greater adhesive strength was observed for those gels with the higher polymer content. The B-Ald hydrogels exhibited adhesive strength values from 40 to 700 kPa for polymer contents of 10, 20, 33 and 50 wt%, respectively. The E-Ald hydrogels exhibited adhesive strength that increased with increasing polymer content, with adhesive strength ranging from 16 to 300 kPa for polymer contents of 10 to 50 wt%, respectively. The E-Ald hydrogels were found to be weaker than the B-Ald hydrogels consistent with the elasticity data (G^* of their relaxed state (before swelling)). Nonetheless, both hydrogels exhibited sufficient adhesive strength for application as ocular adhesives.

Finally, the hydrogels were able to successfully seal full thickness *ex vivo* 4.1 mm corneal wounds as well as or better than a single suture, which is the current standard of care. As shown in Figure 6b, wounds closed by either sutures, the B-Ald hydrogel, or the E-Ald hydrogel were able to withstand intraocular pressures (IOP) much higher than 12 mmHg, which is the average intraocular pressure of the human eye. The hydrogel adhesive precursors are applied as a viscous liquid for *in-situ* crosslinking, and, thus, the material flows to form a smooth surface covering the wound. Of the adhesives studied, the B-Ald hydrogels withstood the highest applied pressure compared to the E-Ald hydrogels. The stability of treated wounds exposed to higher than average intraocular pressure values is

especially important because high intraocular pressure spikes are induced by physiologically relevant events such as coughing and sneezing (up to 60 mmHg). Although both sutures and hydrogel adhesives can secure the wound under physiologically relevant conditions, it is important to note that the failure mechanisms between the two are different. For sutures, the wounds will start to leak at a moderate or high pressure but will not be permanently re-opened unless the pressure is high enough to break the suture or tear the ocular tissue holding the sutures in place. In contrast, hydrogel adhesive failure is irreversible at the point of leakage. As a consequence, the adhesive must ideally be able to withstand intraocular pressures well above the physiologically relevant range (e.g., IOP > 100 mm Hg) before failure. The B-Ald and E-Ald hydrogel adhesives tested in this study meet this important requirement.

The composition, performance, and sealing mechanism of several different adhesive formulations has been recently reviewed.^{3,7} The hydrogel adhesives described herein are synthetic biomaterials; as such, the opportunity exists to optimize the chemical structure and resulting material properties based on design requirements for the intended application. With regards to adhesives for repairing corneal wounds, several hydrogel-based adhesive formulations have been reported recently. For example, the B-Ald and E-Ald adhesives, described above, perform as well as dendritic photocrosslinked adhesives, composed of PEG, succinic acid, and glycerol, in an *ex vivo* 4.1 mm full thickness corneal laceration model.²² Corneal wounds have also been repaired with adhesives produced from bio-derived polymers such as hyaluronic acid and chondroitin sulfate. A photocrosslinked hyaluronic acid adhesive has been used successfully to repair full thickness 3 mm linear and stellate rabbit corneal wounds *in vivo*.¹² An adhesive composed of chondroitin sulfate succinimidyl succinate crosslinked with a star poly(ethylene glycol) amine was shown to seal corneal wounds in excess of 200 mm Hg in *ex vivo* full thickness 3 mm porcine corneas.²⁰ Finally, an adhesive prepared from mixing chondroitin sulfate aldehyde and poly(vinyl alcohol-co-vinylamine) was used to secure *ex vivo* full thickness 3 mm linear rabbit corneal lacerations up to a pressure of 101 mmHg.¹⁵ Due to the potential for reduction in suture-related complications, the ease of use, and the ability to withstand high intraocular pressures, the advantages of applying a hydrogel adhesive to repair a corneal wound are clear.

Conclusions

In this study, hydrogel adhesives are successfully formed from homobifunctional PEG polymers with either propionaldehyde, butyraldehyde, or 2-oxoethyl succinate functionalities cross-linked with a peptide-based dendron Lys₃Cys₄. Upon mixing, these macromers cross-link within minutes to form transparent hydrogels that rapidly swell to equilibrium in aqueous media. The physical and mechanical properties of these hydrogels before swelling are strongly dependent upon initial polymer content. The P-Ald hydrogels are much weaker than the B-Ald and E-Ald hydrogels and degrade within hours of cross-linking. The degradation times of B-Ald and E-Ald hydrogels are 1.5 weeks and 24 weeks, respectively. Upon mixing, the cysteine-terminated Lys₃Cys₄ dendron reacts with the B-PEG to form a thiozolidine linkage, which is susceptible to hydrolysis.⁶⁹ The E-Ald hydrogels are cross-linked by the formation of a thiozolidine linkage which spontaneously rearranges to form the more stable pseudoproline linkages. As a result, these hydrogels are relatively slow to degrade due to the stability of pseudoproline cross-links with its amide bond in comparison to the reversible thiazolidine linkage.⁵¹

Further evaluation of the B-Ald and E-Ald hydrogels shows that the hydrogels are not cytotoxic to rabbit corneal fibroblasts but show moderate toxicity to NIH-3T3 fibroblasts at high polymer concentrations. The constituent macromers are not cytotoxic to rabbit corneal fibroblasts and NIH-3T3 fibroblasts at low concentrations. The adhesive strength of B-Ald

and E-Ald hydrogels is also dependent upon hydrogel polymer content with higher polymer content affording greater adhesive strength. In addition, all of these hydrogel adhesives can be used to close full-thickness *ex vivo* 4.1 mm central corneal lacerations well enough to withstand intraocular pressures as high as or higher than those of wounds closed with a single suture. These results demonstrate the feasibility of these hydrogel adhesives for cornea wound repair. The mechanical properties and degradation times can be tuned and, therefore, may be designed to suit a variety of wounds, ranging from quick-healing corneal incisions to slow-healing corneal transplants. Our research, along with that of others, will lead to the development of application-specific hydrogel adhesives for the repair of a variety of ocular wounds with the potential for reducing complications such as secondary trauma, astigmatism, and infections.

Acknowledgments

Funding for this project was provided, in part, by the National Institutes of Health (EY013881) and Boston University.

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Figure 1.

Chemical structures of the peptide-based Lys₃Cys₄ (a) and functionalized poly(ethylene glycol) macromers used for the preparation of adhesive hydrogels: (b) PEG-propionic aldehyde (P-Ald PEG), (c) PEG-butyric aldehyde (B-Ald PEG), and (d) PEG-ester aldehyde (E-Ald PEG). Structures of hydrogel cross-links formed by reaction of the peptide-based Lys₃Cys₄: with functionalized PEGs: the cysteine terminus of the Lys₃Cys₄ reacts with the aldehyde groups of P-Ald PEG or B-Ald PEG to form thiazolidine linkages (e) or with the aldehyde of E-Ald PEG to form a thiazolidine linkage that rearranges by nucleophilic attack at the ester to form a pseudoproline linkage (f).

Figure 2.

Representative photograph of the hydrogel formed by the reaction of the E-Ald PEG with the peptide-based Lys₃Cys₄ dendron taken after 24 hours of swelling (from left to right: 10, 20, 33, and 50 wt% hydrogels). All three hydrogel systems were similar in appearance and optically transparent.

Figure 3. Swelling characteristics of hydrogels: (a) swelling ratio (Q) as a function of initial polymer content (percent by weight) and (b) relationship between polymer volume fraction in the swollen state ($v_{2,s}$) and as-prepared or relaxed state ($v_{2,r}$) (values are mean $\pm$ standard deviation, $n = 3$, * indicates $p < 0.05$ (B-Ald vs. E-Ald)).

Figure 4.

Mechanical properties of P-Ald, B-Ald, and E-Ald hydrogels as a function of initial polymer content (percent by weight) in the “as-prepared” or relaxed state (E_R) (a, c) and after equilibrium swelling (b, d): compressive modulus of relaxed (E_R) and swollen (E_S) hydrogels (a, b); shear modulus of as-prepared (G_R) and swollen (G_S) hydrogels (c, d) (values are mean $\pm$ standard deviation, $n = 3$, * indicates $p < 0.05$ (B-Ald vs. E-Ald)).

Figure 5.

Viability of rabbit corneal fibroblasts (a, c) and NIH-3T3 fibroblasts (b, d) upon exposure to: the constituent macromers (Lys₃Cys₄ dendron, B-Ald PEG or E-Ald PEG) at concentrations of 0.05, 0.5, and 5 wt% for 3 hours (a, b); whole E-Ald and B-Ald hydrogels of various polymer contents for 24 hours (c, d). Values are mean $\pm$ standard deviation, n = 3.

Figure 6.

(a) Adhesive strength of E-Ald and B-Ald hydrogels as a function of polymer content (percent by weight; values are mean $\pm$ standard deviation, $n = 3$, * indicates $p < 0.05$). (b) Pressure at adhesive failure for *ex vivo* rabbit cornea wounds sealed with a single suture, B-Ald or E-Ald hydrogels (50% by weight) cross-linked with peptide dendron Lys₃Cys₄ ($n = 4$).

Table 1

Optical properties of P-Ald, B-Ald, and E-Ald hydrogels in comparison to those of the human cornea as reported in the literature (refractive index,⁷⁰ transmission of visible light⁷¹). The refractive index (n) and percent transmission of visible light (T) were determined for hydrogel samples in the as-prepared or relaxed and swollen states for initial polymer contents ranging from 10% to 50% (by weight). The degradation time (t_d) for these hydrogels was also determined. N/A = degraded before measurements were taken.

	P-Ald		B-Ald		E-Ald		Cornea
	Relaxed	Swollen	Relaxed	Swollen	Relaxed	Swollen	
n	1.34 – 1.37	N/A	1.34 – 1.37	1.34	1.34 – 1.37	1.34	1.40, 1.38
T (%)	86 – 91	N/A	86 – 91	91	86 – 91	91	93
t_d	3 hours $\pm$ 30 min		1.5 weeks $\pm$ 1 day		24 weeks $\pm$ 4 days		---