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ScienceDirect
Biomaterials 28 (2007) 5205–5214
Biomaterials
www.elsevier.com/locate/biomaterials

Leading Opinion

Designing hydrogel adhesives for corneal wound repair ☆

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Received 22 June 2007; accepted 27 August 2007
Available online 21 September 2007

Abstract

Today, corneal wounds are repaired using nylon sutures. Yet there are a number of complications associated with suturing the cornea, and thus there is interest in an adhesive to replace or supplement sutures in the repair of corneal wounds. We are designing and evaluating corneal adhesives prepared from dendrimers—single molecular weight and highly branched polymers. We have explored two strategies to form these ocular adhesives. The first involves a photocrosslinking reaction and the second uses a peptide ligation reaction to couple the individual dendrimers together to form the adhesive. These adhesives were successfully used to repair corneal perforations, close the flap produced in a LASIK procedure, and secure a corneal transplant.

Keywords: Cornea; Wound healing; Hydrogel; Adhesive; Ophthalmology

1. Introduction

Repair of wounds after traumatic or surgical injury is of significant clinical and research importance. The cornea of the eye is one such site where tissue remodeling after injury is critical since the cornea serves an important role in refracting and focusing light rays necessary for clear vision. The cornea possesses the unique characteristics of an orderly arrangement of stromal collagen fibrils and a lack of blood vessels that result in transparency. Inadequate healing of the cornea after injury can result in decreased or loss of vision. Consequently, new surgical materials, instruments, and clinical procedures are being developed to improve patient care. My laboratory designs, prepares, and evaluates new polymer-based hydrogel sealants for the repair of corneal wounds.

Corneal wounds arise from surgical procedures (e.g., transplants, incisions for cataract removal and intraocular lens implantation, laser-assisted in situ keratomileusis), infections (e.g., ulcers), and traumatic injury (e.g., lacerations, perforations). Today, these wounds are repaired using nylon sutures. Depending on the pattern and extent of injury in a corneal wound, multiple sutures are often needed to realign the edges of damaged tissue in an effort to restore the structural integrity of the cornea. Yet, sutures are not ideal because the suture material does not actively participate in healing and the procedure is inherently invasive [1–4]. The specific drawbacks of using sutures include the following: (1) placement of the sutures inflicts additional trauma to corneal tissue, especially when multiple passes are needed; (2) sutures can act as a nidus for infection and incite corneal inflammation and vascularization. With persistent inflammation and vascularization, the propensity for corneal scarring increases; (3) corneal suturing often yields uneven healing resulting in a regular or an irregular astigmatism; (4) sutures are also prone to becoming loose and/or broken postoperatively and require additional attention for prompt removal; (5) sutures require removal by an ophthalmologist, often months after the operation. Each time a suture is removed, trauma occurs and there is a new opportunity for infection; and (6) suturing requires an acquired technical skill that can vary

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0142-9612/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2007.08.041

widely from surgeon to surgeon, thus influencing the time and success of the operation. Consequently, there is clinical interest in an adhesive to replace or supplement sutures in the repair of corneal wounds and—in fact—in all ophthalmic wounds.

It is estimated that globally more than 12 million procedures per year use nylon sutures to close ocular wounds, including an estimated 2 million corneal wounds each year in the US. There are precedents for the use of adhesives. For example, cyanoacrylate glues were reported in the 1960s by Webster et al. for the repair of corneal perforations [5]. However, these glues have limitations with regard to their ease of application and effectiveness. A number of complications have been reported such as cataract formation, corneal infiltration, granulomatous keratitis, glaucoma, and retinal toxicity [6–14]. Cyanoacrylate glues are used "off-label" but have proven to be an effective therapeutic option in certain ophthalmic settings such as sealing small corneal perforations (1 mm) and preemptive treatment of progressive corneal thinning disorders [5,7,15–19].

An effective polymer adhesive for repairing corneal wounds must meet a number of design requirements. An ideal adhesive should: (1) adhere to the moist corneal surface and seal the wound to withstand high intraocular pressures (>80 mmHg); (2) possess rheological properties that allow for controlled and rapid placement (viscosity <100 cP); (3) rapidly cure to seal the corneal wound in a controlled manner (<30 s); (4) quickly restore the intraocular pressure (<24 h); (5) maintain the structural integrity of the eye; (6) possess a refractive index matching that of the native cornea (1.42); (7) possess solute diffusion properties favorable for normal corneal healing ($>2\times10^{-7}$ cm^2/s for small molecules/nutrients); (8) be biocompatible; (9) be more elastic than corneal tissue so as to disfavor formation of an astigmatism during healing; (10) provide a microbial barrier (2–3 days); and (11) be bioabsorbed or exuded from the wound on a time scale consistent with tissue regeneration (days to months depending on the application).

2. Dendrimers

To meet these design requirements for an adhesive, we need a polymer and resulting crosslinked network that can be tuned, both at the molecular and macroscopic scales. A polymer type that lends itself to optimization due to precise control of composition, and tunable properties is a dendrimer. Dendrimers are unique macromolecules and quite different than the more common linear polymers. A schematic of a linear and a dendrimer polymer are shown in Fig. 1. Dendrimers are highly branched polymers possessing three main structural zones consisting of a central core, internal branching layers, and peripheral groups [20–28]. The branched structure of dendrimers affords a globular, three-dimensional macromolecular shape with a multitude of end groups. A linear polymer

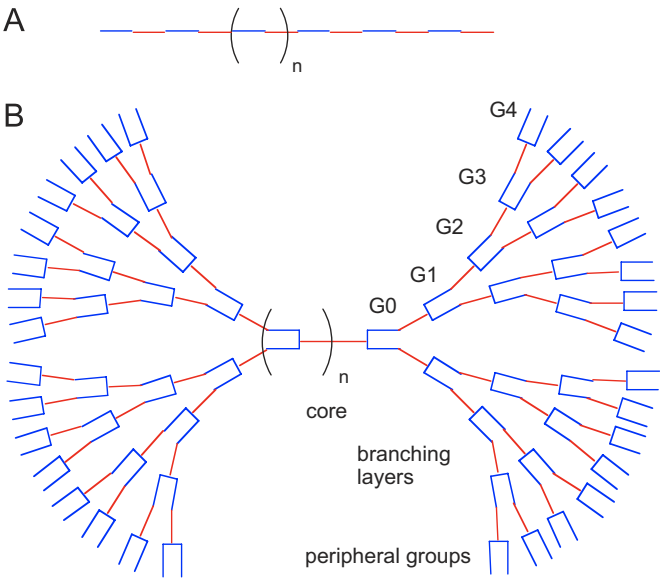


Fig. 1. Schematic of a linear (A) and dendrimer (B) polymer. The three main structural zones of the dendrimer consisting of the central core, internal branching layers, and peripheral groups are labeled.

does not possess this globular structure in solution and has only two end groups. As shown in Fig. 1, the repeat unit in the dendrimer is branched. Each layer in the dendrimer is termed a generation (G) and thus as a dendrimer becomes larger it has a higher number of generations (e.g., G1 vs. G4). Within the structure, a single branch is called a dendron. Additionally, the synthesis and the choice of monomers are highly flexible, since these macromolecules are synthesized in a repetitive manner by either a divergent [29–32], (from core to periphery) or convergent [28,33–36] (from periphery to core) approach. An illustration of a divergent synthetic approach is shown in Fig. 2, where a monomer is added to core using a series of step-wise coupling and deprotection reactions. Importantly, since these polymers possess narrow molecular weight distributions, unlike most polymers, we can correlate a specific biological response, physical property, or rheological property to an exact structure. Thus, we hypothesized that dendritic polymers would provide an opportunity to synthesize, characterize, evaluate, and optimize an ophthalmic tissue adhesive.

Our specific research interest is in degradable and biocompatible dendrimers. We have reported the synthesis and characterization of polyester, polyester-ether, and polyamide dendrimers and dendrons composed of biocompatible building blocks [37–48]. These dendritic polymers are termed "biodendrimers." Examples of a generation four poly(glycerol-succinic acid) dendrimer terminated with hydroxyl groups ([G4]-PGLSA-OH), a generation three dendritic-linear copolymer terminated with hydroxyl groups (([G3]-PGLSA-OH)$_2$-PEG), and a generation two lysine-cysteine dendron ([G2]-(Lys)$_3$-Cys$_4$) are shown in Fig. 3. Cytotoxicity studies show that the polymers are non-toxic with responses similar to non-treated controls.

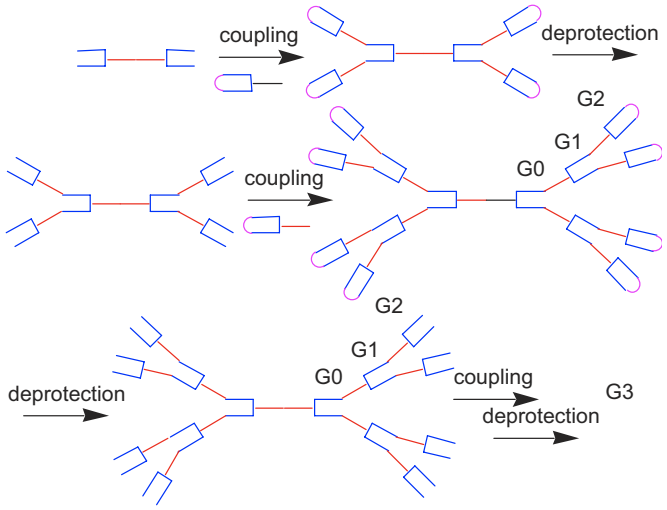


Fig. 2. Divergent synthesis of a generation three dendimer showing the step-wise coupling and deprotection reactions. The monomer consists of three parts—the red portion for coupling to a growing dendrimer, a blue portion which has two potential coupling sites which is capped by a pink protecting group. After the monomer is coupled, the protecting group is removed, and the monomer is added again to create the large dendrimer. This process is repeated until a specific dendrimer generation is prepared.

These data are consistent with the literature reports [49,50] that describe low toxicity with dendritic polymers possessing OH, CO_2H, and PEG end groups. Positively charged groups such as amines generally demonstrate dose-dependent toxicity.

2.1. Crosslinking strategies to adhesives

Subsequent crosslinking of these dendritic macromolecules affords hydrogel adhesives. The crosslinked hydrogel adhesives are transparent, pliable, and soft. A photograph of one such hydrogel is shown in Fig. 4. Hydrogel networks can be formed using two strategies. In the first approach, the end groups of the dendritic macromolecule are modified to contain an acrylate or other free-radical polymerization group [39]. Upon exposure to visible light the acrylated-modified dendritic macromolecule, which is dissolved in aqueous solution containing a small quantity of a photoinitiating system, crosslinks to form a hydrogel (Fig. 4). This visible photoinitiating system comprises eosin Y, 1-vinyl-pyrrilidinone, and triethanol amine. Photolysis of the solution using an argon ion laser (514 nm) initiates the free radical polymerization of the methacrylate moieties on the dendritic polymer. This initiating system has been previously used and shown to be non-toxic [51–54]. This in situ photocrosslinking approach which delivers a liquid polymer to a site followed by solidification of the polymer via light to form a three-dimensional hydrogel network is an exciting modality being explored by many groups [55–57].

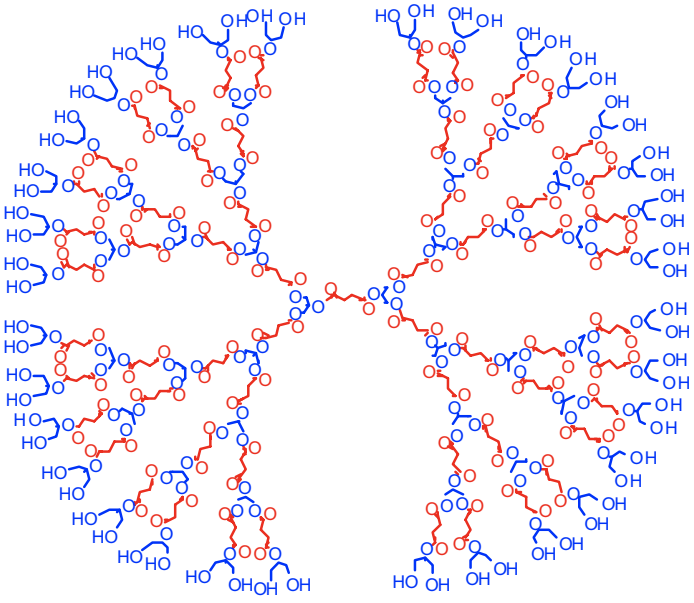
Alternatively, the end groups of the dendritic macromolecule are decorated with nucleophiles and subsequently reacted with another polymer containing electrophiles. Of particular interest to us, is the identification of nucleophile–electrophile crosslinking chemistry which would occur rapidly at 37 °C under neutral aqueous conditions without the generation of side-products. Moreover the reaction needs to be chemoselective and possess a high tolerance to a range of other chemical functionalities (e.g., amines, thiols, carboxylates) which may be present under the conditions of crosslinking. Consequently, we selected a peptide ligation reaction and have recently used such chemistry to prepare a hydrogel [46]. Specifically, we mixed aqueous solutions of a dendron containing *N*-terminal cysteines and a PEG-dialdehyde (PEG-DA) to afford a crosslinked network via formation of thiazolidine linkages throughout the hydrogel (Figs. 3 and 4) [46]. This mild procedure involving a thiol and amine reacting with an aldehyde has been applied successfully to the synthesis of a variety of proteins [58–62]. These two crosslinking strategies have been used to repair a variety of corneal wounds, as described below.

2.2. Corneal wounds

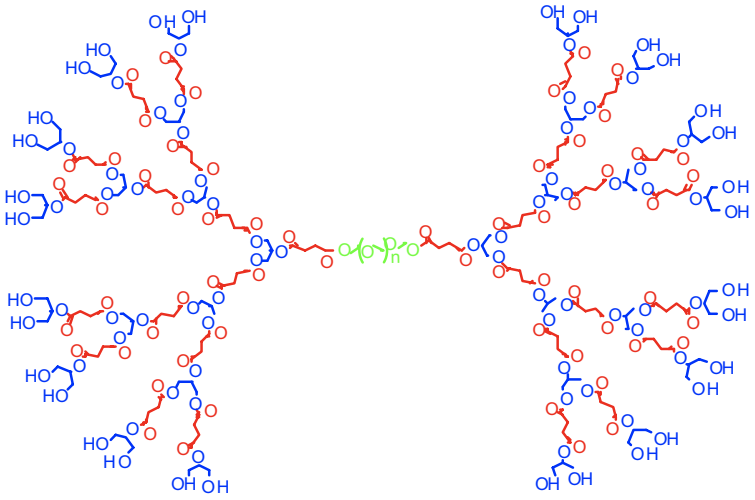
Repairing corneal lacerations, sealing clear corneal incisions, securing corneal transplants, and closing LASIK flaps are four ophthalmic indications where the use of an adhesive is advantageous. We will focus our discussion to corneal lacerations and clear corneal incisions. Corneal lacerations which are caused by trauma, infection, or inflammation are an ophthalmic emergency that can lead to loss of vision. A clear corneal incision is the wound made during a cataract procedure. Today, surgeons break up and remove the cataract using ultrasound energy and implant a synthetic intraocular lens, all through this incision in the cornea. In the following text, we will describe two of the dendritic macromolecule adhesive formulations we have developed and their use to repair corneal wounds.

2.3. Photochemical crosslinkable adhesives

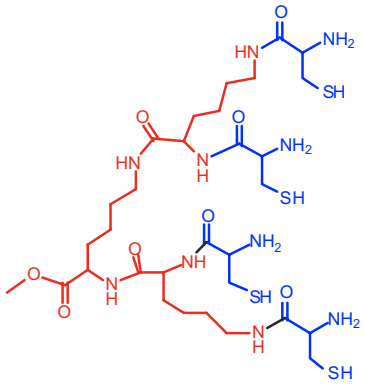
We first examined a photocrosslinkable biodendrimer adhesive for repairing 4.1 mm linear corneal lacerations [39,40,63]. The specific polymer used was a dendritic linear copolymer composed of PEG, glycerol, and succinic acid like the one shown in Fig. 3. The polymer was modified to contain terminal methacrylate (MA) groups and dissolved in a neutral aqueous solution. Upon exposure of ([G1]-PGLSA-MA)$_2$-PEG to visible light a crosslinked network is formed. A keratome knife was used to create 4.1 mm full-thickness linear incisions in the central cornea of enucleated human eyes. In this ex vivo experiment, the wound was closed using either three interrupted 10-0 nylon sutures in a standard 3-1-1 suturing configuration or the photocrosslinkable dendritic macromolecule. Application of 20 μL of the photocrosslinkable adhesive to the laceration followed by irradiation with visible light rendered the hydrogel which sealed the wound (Fig. 5; argon ion laser,



[G4] - PGLSA -OH



([G3] - PGLSA-OH)$_2$ - PEG



[G2]- (Lys)$_3$-Cys$_4$

Fig. 3. Chemical structures of a generation four poly(glycerol-succinic acid) dendrimer ([G4]-PGLSA-OH), a generation three dendritic-linear copolymer (([G3]-PGLSA-OH)$_2$-PEG), and a generation two lysine-cysteine dendron ([G2]-(Lys)$_3$-Cys$_4$).

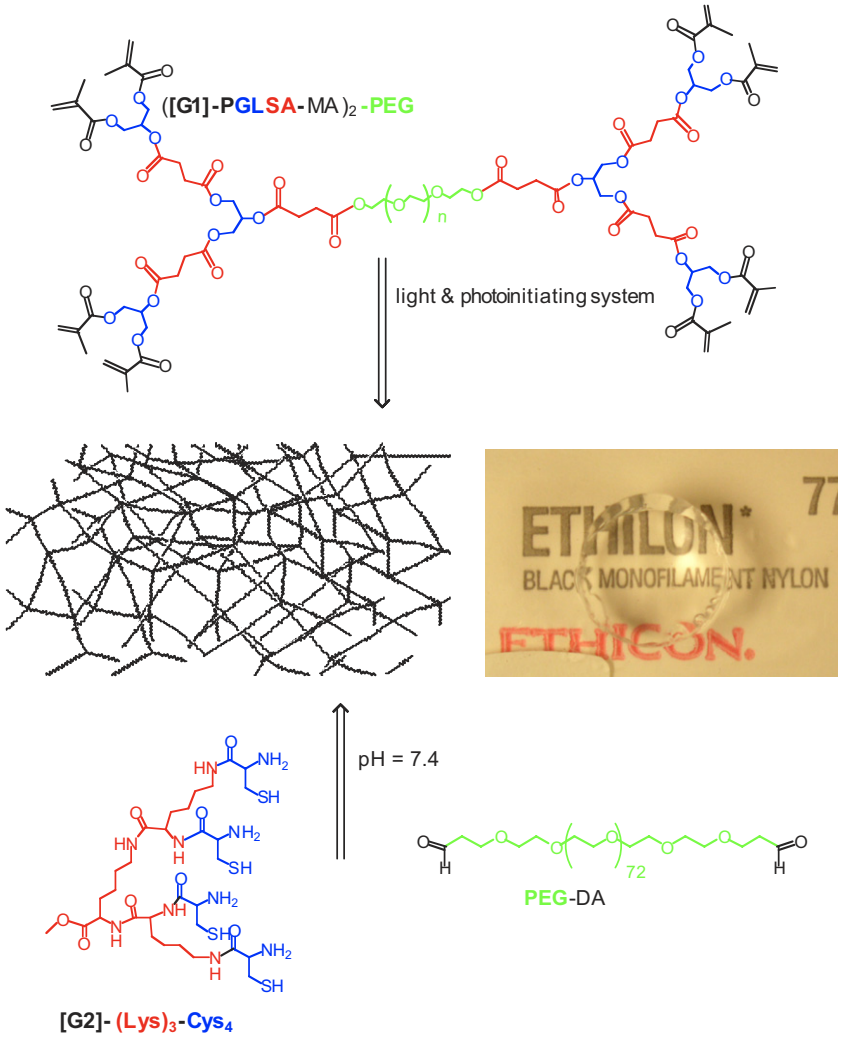


Fig. 4. Schematic of the two crosslinking strategies: (top) photocrosslinking reaction and (bottom) nucleophile–electrophile reaction.

Fig. 5. Photographs of the closure procedure for a 4.1 mm full thickness corneal laceration. (left) Placement of the adhesive solution on the wound. (middle) Photocrosslinking of the solution to form the adhesive. (right) Sealed corneal laceration.

514 nm, 200 mW, 1 s exposures; 60 s total irradiation time; the polymer solution contained ethyl eosin in 1-vinyl pyrrolidinone and TEA as photoinitiator and co-catalyst, respectively). The eye globes were then inflated with saline solution while the intraocular pressure was monitored via a pressure transducer until fluid leaked from each eye. Using this procedure an initial study was conducted with different generations of the dendritic macromolecule (i.e., ([G0]-PGLSA-MA)$_2$-PEG, ([G1]-PGLSA-MA)$_2$-PEG, ([G2]-PGLSA-MA)$_2$-PEG, and ([G3]-PGLSA-MA)$_2$-PEG) and it was found that the adhesive formed from ([G1]-PGLSA-MA)$_2$-PEG performed the best. This adhesive gave a tight seal and was easily applied and subsequently crosslinked. The smaller generation dendrimers did not crosslink effectively under the operating conditions to give a leak-tight seal and the larger dendrimer gelled, but then delaminated from the surface without forming an adequate seal. For globes that received a linear incision in the experiment, the mean leaking pressure was 79 mmHg for the suture group and 110 mmHg for the ([G1]-PGLSA-MA)$_2$-PEG adhesive group. Control globes that received only dendritic polymer but no photocrosslinking or

photocrosslinking alone did not seal the wound. The difference in leakage pressures was significant relative to 34 mmHg, the IOP under stressful physiologic conditions, e.g., coughing and valsalva maneuver. The adhesive withstood pressures well above normal physiological intraocular pressure of about 12 mmHg. This adhesive was also effective for repairing 4.1 mm stellate corneal perforations, closing the flap produced in a LASIK procedure, and securing a corneal transplant ex vivo [63–65].

With these successes, we initiated an in vivo study to compare the clinical and histological healing response of corneal lacerations repaired by either traditional sutures or the light-activated dendritic adhesive ([G1]-PGLSA-MA)$_2$-PEG. In vivo corneal wound healing studies have been previously performed on primates [66,67], cats [68], dogs [69], rats [70], chickens, and rabbits [71,72]. Rabbits have been the most commonly used animals for these types of in vivo experiments [71,72], however, the rabbit cornea heals rapidly compared to the human eye and the rabbit cornea does not possess a Bowman's membrane. Thus, we selected the chicken model (White Leghorn Chicken; *Gallus gallus domesticus*) to test the efficacy of this adhesive since the chicken cornea is similar to the human cornea and the healing response is similar to humans [73,74]. It is only recently that the chicken model is becoming a preferred model [73,75,76], and the Commission of the European Communities has recently recognized the chicken eye as a preferred model for assessing eye irritation [77]. After the 4 mm full-thickness linear corneal wounds were made in the right eye of white leghorn chickens, half of the animals, 30, received approximately 20 μL of the ([G1]-PGLSA-MA)$_2$-PEG adhesive solution and the other half, 30, received 3 interrupted 10-0 nylon sutures. Healing of corneal perforations following treatment was evaluated clinically for up to 28 days in the 60 chickens. At 6 h and 1, 3, 4, 5, 6, 7, 14, 21, and 28 days after surgery, slit-lamp examination of corneal healing and Seidel tests for wound leaking were performed. No evidence of a toxic response to the procedure or the biodendrimer adhesive was observed during the clinical examinations indicating good biocompatibility. Animals were sacrificed at days 1, 3, 7, and 28. Histological examination was performed to determine the time course and extent of corneal healing. All wounds were confirmed to be Seidel positive and not self-sealing incisions. Histological studies performed after post-operative day zero also confirmed the lacerations were not self-sealing. The anterior chambers quickly refilled, following the application and photopolymerization of ([G1]-PGLSA-MA)2-PEG, or suture placement. Seidel tests confirmed that by post-operative day 2, all of the adhesive and suture treated eyes were Seidel negative—that is the wound was sealed and did not leak (Fig. 6). More corneal haze was evident in the sutured group on post-operative day one with subsequent scarring evident at all later days when compared to the adhesive group. By post-operative day 1, all anterior chambers had formed. The adhesive was visibly present in most of the treated eyes on post-operative days 1–7, but had disappeared by day 14.

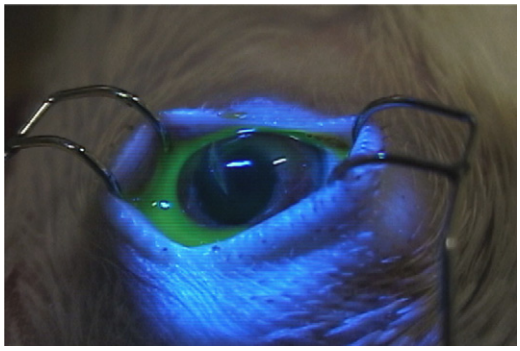


Fig. 6. Seidel test confirming the closed corneal laceration.

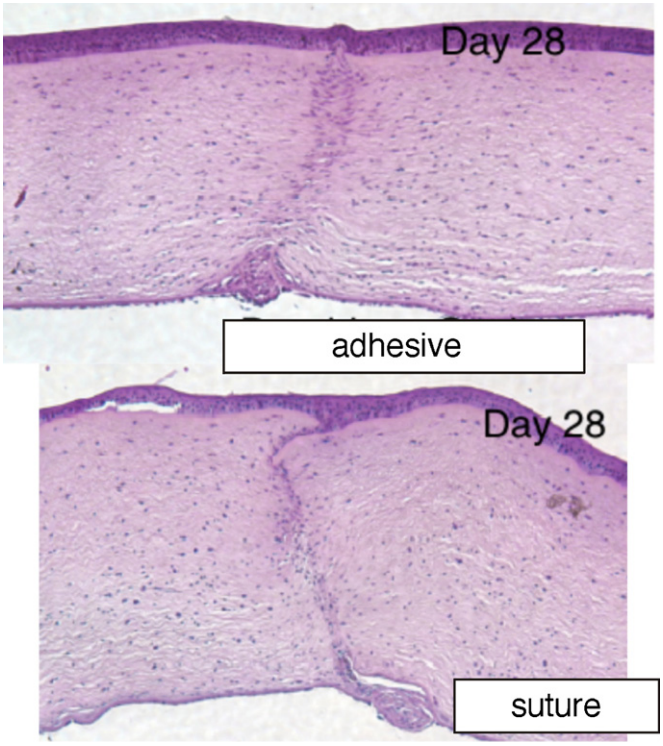


Fig. 7. H & E stains of the repair chicken cornea after treatment with a dendrimer adhesive (top) and suture (bottom).

Histological studies were performed to determine the time course and extent of corneal healing on all chicken eyes at 1, 3, 7, or 28 days after application of the adhesive or sutures. Histological sections at day 28 for the adhesive and suture are shown in Fig. 7. The photocrosslinkable ([G1]-PGLSA-MA)$_2$-PEG adhesive completely sealed 97% (28/29) of the linear lacerations on post-operative day 1 and all wounds were Seidel negative by post-operative day 2. Initially, the sutured wounds appeared to form a more stable wound histologically at day 3. However, between days 3 and 7 the wounds from both groups looked similar except that the interface between the epithelial and stromal layers was more uniform with the adhesive. At day 28, the wounds sealed with the adhesive appeared histologically

more complete. A more uniform stromal layer, no detachments between the stromal and epithelial layer, and no overlapping of the Bowman's layer was observed compared to the wounds treated with sutures.

2.4. Peptide ligation crosslinkable adhesives

In the course of performing these in vitro, ex vivo, and in vivo experiments, it became evident that an adhesive that did not require light for crosslinking and formation would also be of interest to clinicians. Ease of use, simplicity of application procedure, and reducing procedural risks are three important milestones to achieve, when the goal is to translate basic research to the clinic. As mentioned above, it was important to identify mild crosslinking chemistry which occurs rapidly in aqueous solution at pH 7 and at 37 °C and is chemoselective. Thus, we are investigating the use of a thiazolidine linkage which is formed between an *N*-terminal cysteine and an aldehyde; this reaction belongs to a family of peptide ligation reactions [58–62]. For this approach to work, we need a dendritic polymer which contains 3 or more *N*-terminal cysteines and a PEG which contains at least two terminal aldehyde groups. Aldehyde-based adhesives have been explored for ophthalmic use including a condroitin sulfate and a PEG–PLL micelle system as well as there is a commercial product used for cardiovascular surgery [78–80]. Thus, we prepared a G2 lysine-based dendron with terminal cysteines ([G2]-$(Lys)_3$-Cys_4) and a PEG di-aldehyde (PEG-DA) as shown in Figs. 3 and 4. A hydrogel is formed upon mixing an aqueous solution of the two polymers as a result of the thiazolidine linkages.

With this hydrogel formulation in hand, we decided to evaluate this adhesive for sealing clear corneal incisions—the wound made during a cataract procedure. Cataract removal is the most commonly performed ophthalmic surgical procedure, and this number is expected to increase with the aging demographics. At the conclusion of the procedure, this clear corneal incision is either left alone to "self-seal" or closed with nylon sutures. As mentioned earlier, suturing has a number of drawbacks but so does the "self-seal" approach. These risks include leakage and increased endophthalmitis with an occurrence rate of 0.3 [81,82]. To determine whether this hydrogel sealant would secure a clear 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.

For this ex vivo experiment, a 3 mm clear corneal linear incision was made in an enucleated eye. This wound was either left to self-seal, closed using one interrupted 10-0 nylon suture or the hydrogel adhesive. For the hydrogel adhesive, dendron ([G2]-$(Lys)_3$-Cys_4) and PEG-DA were mixed quickly at room temperature and then $\approx$20 μL of the hydrogel adhesive was applied to the incision. Fig. 8 shows a 3 mm clear corneal wound leaking before treatment as well as a sealed wound repaired using the

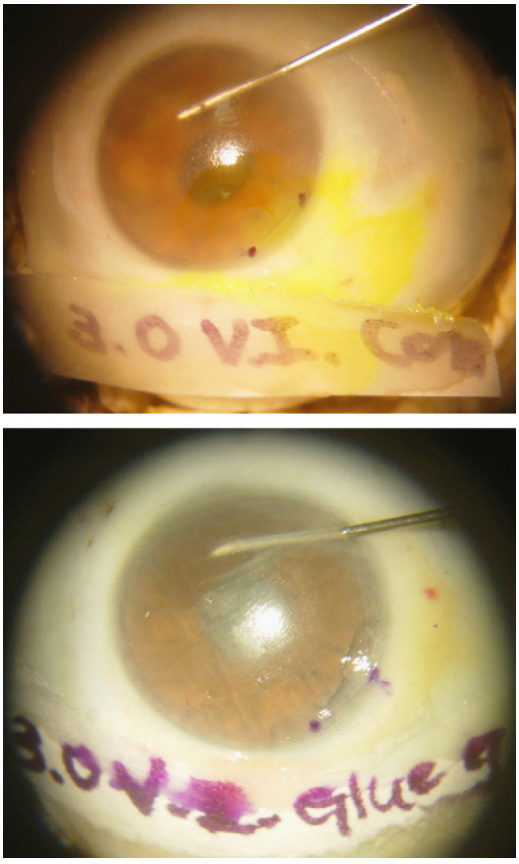


Fig. 8. (Top) A 3 mm clear corneal wound leaking before closure. (bottom) A sealed wound with the dendritic adhesive composed of ([G2]-$(Lys)_3$-Cys_4 and PEG-DA.

hydrogel adhesive. 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 ex vivo 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 adhesive ($n = 8$) was 184 ± 79 mmHg. The incision is not sealed using only the dendron or PEG-DA hydrogel precursors. The larger dendron showed similar results and thus we focused our efforts on the ([G2]-$(Lys)_3$-Cys_4) since this dendron performed well and was easier to synthesize. The hydrogel adhesive secures the clear corneal incision and withstands higher pressures and stresses placed on a wound than conventional suture or self-sealed treated wounds. The procedure with the hydrogel adhesive is facile and requires less surgical time than conventional suturing (4–6 times), does not inflict additional tissue trauma, and does not require additional instruments (e.g., laser) to prepare the crosslinked adhesive. Using this crosslinking strategy, we have also closed LASIK flaps and repaired corneal lacerations. The crosslinked hydrogel adhesive is transparent, adhesive, elastic, hydrophilic, and acts a physical protective barrier to the ocular surface.

3. Conclusion

In summary, in situ polymerizing hydrogel-based adhesives have been developed and evaluated for the repair of corneal wounds. The use of crosslinkable dendritic macromolecules for this application is advantageous since the high level of molecular control enables precise designing and prototyping of the macromolecule as a macromer. Both the photocrosslinking and nucleophile–electrophile crosslinking strategies afford hydrogels that are adhesive, transparent, elastic, hydrophilic, and soft. An advantage of the photocrosslinking approach is the ability to crosslink and adhere tissue only where the clinician directs the light. However, the limitation is the potential risks with ocular damage when using light. The self-gelling approach eliminates the need for light and will crosslink upon placement on the tissue. The specific crosslinking chemistry based on peptide ligation is attractive over previous systems such as N-hydroxysuccinimide (NHS) or malimide because no by-products are generated, the reaction is performed at neutral pH, and the reaction is chemoselective (i.e., only coupling between the correct partners). These hydrogel adhesives can be used alone or in conjunction with a reduced number of sutures to secure the corneal wound interface. The dendritic macromolecules used to form these hydrogel adhesives belong to a class of macromolecules composed of biocompatible building blocks which are highly branched and have multiple end groups. This system enables efficient crosslinking, varied hydrogel properties, and aqueous polymer solutions of the adhesive formulation for application to a wound site.

In my opinion, where are we today? I believe that a corneal adhesive has the potential to change clinical practice and improve patient care. While we do not yet have a corneal adhesive that a clinician can use sitting on the shelf in his/her office, we have made significant progress. Also, we have a better understanding of the performance requirements needed for such an adhesive. Academic and industrial scientists are working on this advancement. Will there be a suitable product on the market within 3–5 years? The answer must be yes—patients need one. As an interdisciplinary team composed of chemists, engineers, and ophthalmic surgeons, we are committed to develop a corneal adhesive for use in the clinic.

Acknowledgments

This work was supported by the NIH. I wish to thank my long-term collaborator Dr. Terry Kim and his fellows who worked on this project Drs. M. Starck Johnson, Pil Jung, Paul C. Kang, Jitek Kim, Johannes Kristinsson, Crystan Middleton, and Andrew Velazques at the Duke Eye Center for their time, surgical expertise, and interest in evaluating tissue adhesives for corneal wound repair. I also thank my graduate students and post-doctoral fellows for their hard work and dedication to this project: Jason Berlin, Michael A. Carnahan, Lovorka Degoricija, Nathanael R. Luman, Abigal Oelker, Kimberly A. Smeds, and Dr. Michel Wathier.

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