



Comparison of conjunctival pedicle flap to corneal fixation strength achieved by Tisseel[®] fibrin glue, ethyl cyanoacrylate adhesive, ReSure[®] hydrogel sealant, and conventional suturing with 8-0 VICRYL[®] ophthalmic suture

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Abstract

Objective: To determine and compare the fixation strength of conjunctival pedicle flaps to cornea achieved via conventional ophthalmic suture and three different adhesive compounds.

Animals Studied: Ex vivo porcine globes.

Procedures: Following a 6 mm wide 500-micron-restricted depth lamellar keratectomy, conjunctival pedicle flaps were secured to the keratectomy site with either 8-0 VICRYL[®] suture or one of three adhesive products, including Tisseel[®] bioadhesive, ReSure[®] synthetic adhesive, or ethyl cyanoacrylate adhesive ($n = 10$ per surgical group). Adhesive application protocol varied by product based upon adhesive biocompatibility. Corneoconjunctival tissues were then harvested, clamped in a tensile testing device, and loaded at a rate of 1 mm/s under video surveillance until the point of failure. Peak load was determined for each test and used to compare fixation strength between samples.

Results: Forty conjunctival flaps were performed, with 6 omitted from evaluation due to dehiscence prior to tensile testing. Of the 34 flaps analyzed, 10 were secured with suture, 10 with cyanoacrylate, 8 with ReSure[®], and 6 with Tisseel[®]. Flaps secured with suture withstood significantly higher applied tensile force compared with cyanoacrylate ($p = .02474$), ReSure[®] ($p = .00000$), and Tisseel[®] ($p = .00002$). Flaps secured with cyanoacrylate withstood significantly greater force than those secured with ReSure[®] and Tisseel[®] ($p = .01194$ and 0.01798 , respectively). There was no significant difference in fixation strength between ReSure[®] and Tisseel[®] glue ($p = .95675$).

Conclusions: Conjunctival pedicle flap fixation using 8-0 VICRYL[®] suture fixation was able to withstand significantly greater maximum tensile force compared to ReSure[®], Tisseel[®], or cyanoacrylate adhesives. Fixation strength

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achieved with cyanoacrylate adhesive was significantly greater than that achieved with ReSure® or Tisseel®.

KEYWORDS

conjunctival flap, cornea, cyanoacrylate, fibrin adhesive, fixation, hydrogel

1 | INTRODUCTION

Ulcerative keratitis is a common ocular surface disease with a prevalence of approximately 0.8% in the general canine population.¹ With appropriate treatment, most corneal ulcers heal uneventfully, but complications including secondary infection and collagenolysis can lead to corneal stromal loss, leaving the cornea weakened and at risk of perforation, a potentially sight threatening condition. Once the cornea has reached a fragile state, surgical interventions to provide tectonic support may become necessary. In veterinary ophthalmic practice, conjunctival flaps and grafts are the most frequently employed surgical procedures to address deep corneal defects.²⁻⁴ The use of conjunctiva as a stabilizing material is well documented, with surgical variations including the conjunctival pedicle flap, bridge flap, conjunctival island graft, conjunctival hood flap, and the 360-degree conjunctival flap, which are all reported to have a high success rate.²⁻¹² Of the described surgical techniques, all exploit the same characteristics that make conjunctiva an appealing autologous transplant. The conjunctiva is redundant in nature and in close proximity to the corneal surface, thus readily available for transfer to the cornea. It is also easily dissected to appropriate size for repositioning over the corneal defect, where it effectively aids in healing through direct provision of structural support and, in the case of flaps, via a rich blood supply.

Securing the conjunctiva to the cornea is typically achieved through suturing, which is technically challenging and can result in prolonged surgical times, increased corneal edema, increased scar tissue, foreign body reaction, abscess, and dehiscence.^{13,14} However, a number of sutureless techniques to affix ocular tissues are utilized or are being explored. Among others, these approaches include the use of synthetic adhesives, bioadhesives, and hydrogel sealants.¹⁵⁻²⁷ Proposed advantages of adhesives over suture, include reduced operative times, watertight closures, decreased foreign body reaction and inflammatory response, faster healing times, and increased ability to induce regeneration of the original tissue architecture.²⁸⁻³² In corneal application there are several reported disadvantages of adhesives, particularly cyanoacrylates. These include

cellular toxicity related to heat generated during adhesive polymerization and formaldehyde release during the degradation process.^{33,34} Foreign body reactions and patient discomfort due to development of a rough surface following polymerization are also reported.³⁵

In this study, we affixed conjunctival pedicle flaps to keratectomy sites in ex vivo porcine globes utilizing four discrete security techniques, including 8-0 ophthalmic suture, cyanoacrylate adhesive, ReSure® hydrogel sealant, and Tisseel® fibrin glue. The aim of the study was to determine and compare the strength of corneconjunctival fixation achieved by these four methods.

2 | MATERIALS AND METHODS

2.1 | Ocular tissue samples

Ex vivo porcine globes were obtained (Animal Technologies Tyler) within 24 h of animal sacrifice for reasons unrelated to this study. The specimens were fresh, never frozen, and stored in a cooler on ice for transport. On the day of arrival, each eye underwent examination by a board-certified veterinary ophthalmologist and resident in training for determination of normal corneal and conjunctival anatomy. Globes with corneconjunctival abnormalities not attributable to expected postmortem findings were excluded from the study.

2.2 | Adhesive and suture materials

Three different classes of adhesives and one suture material were utilized for conjunctival flap fixation in this study. The commercially available fibrin glue, Tisseel® (Baxter) was selected as a representative biologic adhesive. Ethyl cyanoacrylate adhesive (All-purpose Crazy Glue®; Elmers Products) was selected as a representative synthetic glue and ReSure® hydrogel sealant (Ocular Therapeutix) was selected as a polyethylene glycol (PEG)-type adhesive. The ophthalmic suture material utilized was VICRYL® 8-0 (Polyglactin 910 with 5.5 mm 1/2c spatula, MWI Animal Health, Idaho, United States).

2.3 | Study design

Four treatment groups (A–D), each designating a different method of conjunctival pedicle flap fixation, were defined as follows: Group A (Cyanoacrylate, $n=10$ eyes), Group B (ReSure[®], $n=10$ eyes), Group C (Suture, $n=10$ eyes), and Group D (Tisseel[®], $n=10$ eyes). To ensure the use of only fresh tissue samples, to maintain a single surgeon (EV) throughout the experiment, and to accommodate engineering lab availability, the study was carried out on 4 nonconsecutive days. To maintain consistency within treatment group, experimental procedures for each group were performed in their entirety on each of the four study days. Groups A through D were designated to experimental Days 1 through 4, respectively. On the day of arrival, eyes were selected via random drawing for inclusion. Once examined and deemed free of corneconjunctival disease, the eyes were prepared for surgery, which included trimming of the eyelashes and vibrissae, flushing with sterile eye wash, and securing the globes to a foam tabletop operating stage. Surgical loupes with 3.5x magnification were used throughout the surgical procedures.

2.4 | Conjunctival pedicle flap preparation

For each specimen, the conjunctival pedicle flap was dissected first. Starting at the 9 o'clock position approximately 1 mm posterior to the limbus, the donor bulbar conjunctiva was separated from the globe and Tenon's capsule with a combination of blunt and sharp dissection. An approximately 7 mm wide pedicle flap was dissected from the dorsotemporal aspect of the globe, with the pedicle base located at the 12 o'clock position. If a conjunctival "button hole" lesion occurred during dissection, the globe was discarded and replaced with a new specimen.

2.5 | Keratectomy procedure

After conjunctival flap dissection was complete, a 6 mm corneal stromal defect was made via keratectomy to serve as the flap recipient site, as follows. In order to improve tissue sectility during keratectomy, globes were reinflated via intracameral injection of water via 30-gauge needle at the 6 o'clock position. Focal stromal hydration was employed to prevent leaking at the injection site as the needle was withdrawn. A 6 mm diameter punch biopsy (Intervet Inc. Merck Animal Health) outfitted with a 3D-printed (Lulzbot[®] Taz 6, Additive Manufacturing Equipment 3D, LLC) depth restrictor was used to create a 500-micrometer deep corneal defect centered 3 millimeters ventral to the dorsal limbus at the 12 o'clock position. Sharp lamellar dissection using a 6400-beaver blade was used to free the defined corneal button from the underlying stroma (Figure 1). To ensure a consistent surface contact area for the adhesives, a one-millimeter rim of epithelium was then removed around the perimeter of the keratectomy site (Figure 1). This defined perimeter was accomplished using Castroviejo calipers for alignment of an 8 mm punch biopsy (Intervet Inc. Merck Animal Health) surrounding the recipient site. The 1-mm rim of epithelium was lifted with a 6400-beaver blade and peeled or sharply excised from around the graft recipient site. This region served as a delineated moat to prevent adhesive from migrating past this defined point, ensuring that the applied adhesive surface area was uniform between samples. Once the keratectomy bed was prepared, the conjunctival pedicle flap was rotated ventrally to rest within the keratectomy bed without tension (Figure 1). When necessary, the flaps were trimmed to size to reside within and flush with the 6 mm recipient bed border.

2.6 | Conjunctival pedicle flap fixation

A timer was used to record the amount of time, in seconds, required for completion of conjunctival pedicle

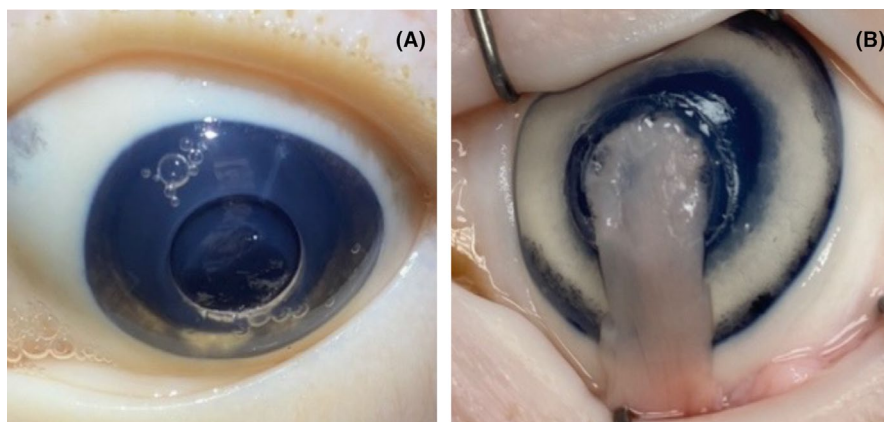


FIGURE 1 (A) 6 mm keratectomy site in ex vivo porcine cornea. (B) Conjunctival pedicle flap resting without tension in a 6 mm recipient keratectomy bed prior to being secured. A 1 mm perimeter of debrided stroma is evident surrounding the flap, where corneal epithelium was removed in order to contain and standardize adhesive application surface area.

flap fixation to the cornea. The timer was started the moment when adhesive was applied to the tissue or in the case of suturing, when the first suture was passed through the conjunctiva. Completion was defined as the time when the adhesive was dry to the touch (confirmed via gently dabbing with a Weck-Cel® spear) or the completion of the final suture. Due to differences in biocompatibility, adhesive application technique varied between groups to mimic potential application in a clinical scenario. Because cyanoacrylate adhesive and ReSure® hydrogel sealant slough during healing, they were placed only over the outer surface interface of the cornea and conjunctiva. The fibrin glue was placed in the same manner, but additionally under the flap within the keratectomy bed as it is intended to incorporate into the host tissue.

Group A (ethyl cyanoacrylate): The corneal and conjunctival surfaces were dried with a Weck-Cel® spear (Intervet Inc. Merck Animal Health, New Jersey, United States). Ethyl cyanoacrylate adhesive was manually applied with a fine-tip paint brush over the corneoconjunctival interface, not extending beyond the delineated moat. The cornea/flap/adhesive interface was allowed to cure until completely dry (confirmed via gently dabbing with a Weck-Cel® spear).

Group B (ReSure®): Similar to group A, the corneal and conjunctival surfaces were dried with a Weck-Cel® spear (Intervet Inc. Merck Animal Health). ReSure® polyethylene glycol adhesive was reconstituted (according to manufacturer guidelines) and manually applied with the commercially provided applicator over the corneal conjunctival interface, not extending beyond the 1-mm delineated moat. The cornea/flap/adhesive interface was allowed to cure until completely dry (confirmed via gently dabbing with a Weck-Cel® spear).

Group C (VICRYL®): The conjunctival pedicle flaps were secured to the recipient site using a simple interrupted suture pattern of 8-0 VICRYL® suture material. Five simple interrupted sutures were placed at the 2:00, 4:00, 6:00, 8:00, and 10:00 o'clock positions. For consistent depth of corneal suture placement, suture was first passed through the conjunctival flap entering the cornea at the deepest aspect of the recipient bed and secured with four throws.

Group D (Tisseel®): As with groups A and B, the corneal and conjunctival surfaces were dried with a Weck-Cel® spear. The fibrin glue was prepared according to the manufacturer. Briefly, the aprotinin (Fibrinolysis Inhibitor Solution) was transferred into the vial containing the freeze-dried Sealer Protein Concentrate using the sterile reconstitution components. The vials were gently mixed to ensure that the freeze-dried material was completely soaked. The vials were then placed into a manufacturer

approved incubation device pre-warmed to 37 degrees Celsius. Vials were stirred manually until all the sealer protein concentrate was dissolved. In the same fashion, the thrombin solution was reconstituted with the calcium chloride solution.

The sealer protein solution and thrombin solution were then combined and applied to the keratectomy bed and over the corneoconjunctival interface using the Duploject® (Baxter, California, United States) application system provided, taking care to ensure that the glue did not extend past the 1 mm delineated moat. As a biologic media that incorporates into host tissue, the fibrin glue was placed directly in the keratectomy bed under the flap, as well as over the surface of the flap. The adhesive was allowed to clot until solidified (confirmed via gently dabbing with a Weck-Cel® spear).

2.7 | Harvesting the cornea

Following conjunctival flap fixation, the pedicle flap base was transected from the bulbar aspect of the globe using tenotomy scissors. The entire cornea including the attached conjunctival flap interface was then sharply excised from the globe at the limbus (Figure 2). The tissue samples were then transported to the Virginia Tech College of Engineering, Center for Injury Biomechanics Laboratory in a humidity-controlled container for tensile testing.

2.8 | Tensile testing

Axial tension tests were conducted on isolated corneoconjunctival specimens at room temperature. The testing device was configured with two motor driven linear stages (Parker Daedal, MX80S), which were operated with a multi-axis controller (Parker, ACR9000) and motor driver (Parker, ViX).³⁶ Each of the linear stages were instrumented with a single-axis load cell (Interface, WMC-5), accelerometer (Endevco, 7264B-2000G), and potentiometer (Firstmark Controls) (Figure 3). A previously developed testing procedure was used to maintain consistency in regard to the initial specimen preload and alignment.³⁶ First, the top grip assembly was positioned on a flat surface. The sample was rested on the top grip so that the long axis of the sample was in-line with the load train. Once aligned, the top portion of the specimen, that is, the cornea, was clamped into the top grip. Then, the top grip assembly was reattached to the testing device, allowing the conjunctival pedicle to hang under its own weight. This allowed for a proportionally consistent tensile preload

FIGURE 2 Gross image of a harvested conjunctival pedicle flap secured to cornea with (A) *ethyl* cyanoacrylate adhesive, (B) ReSure[®] hydrogel sealant, (C) Tisseel[®], and (D) 8-0 VICRYL suture material.

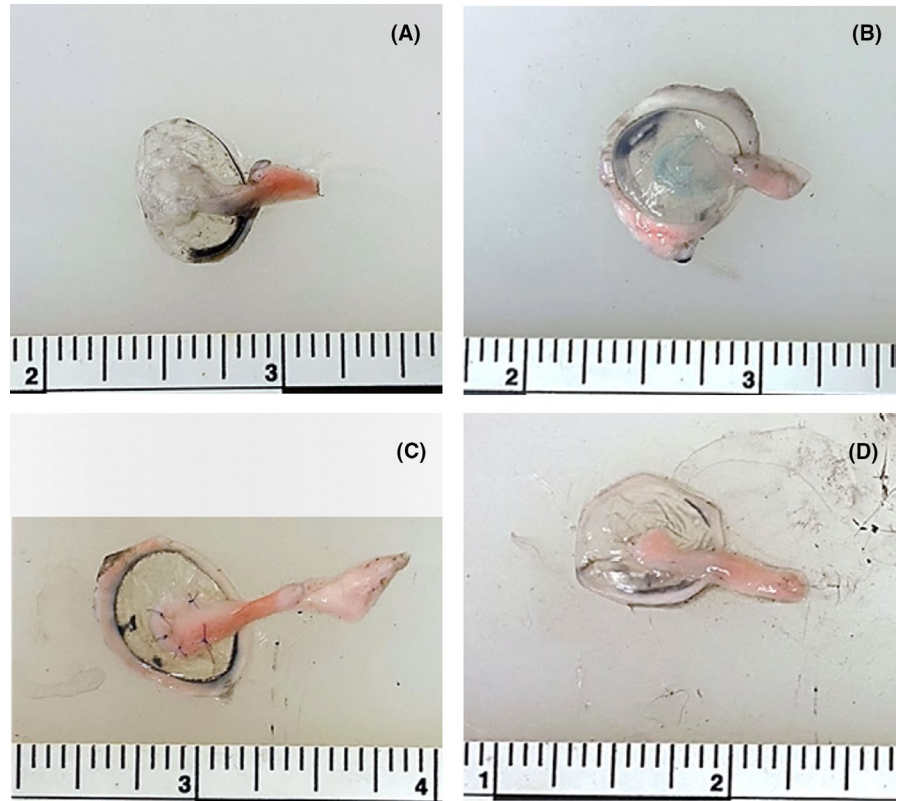
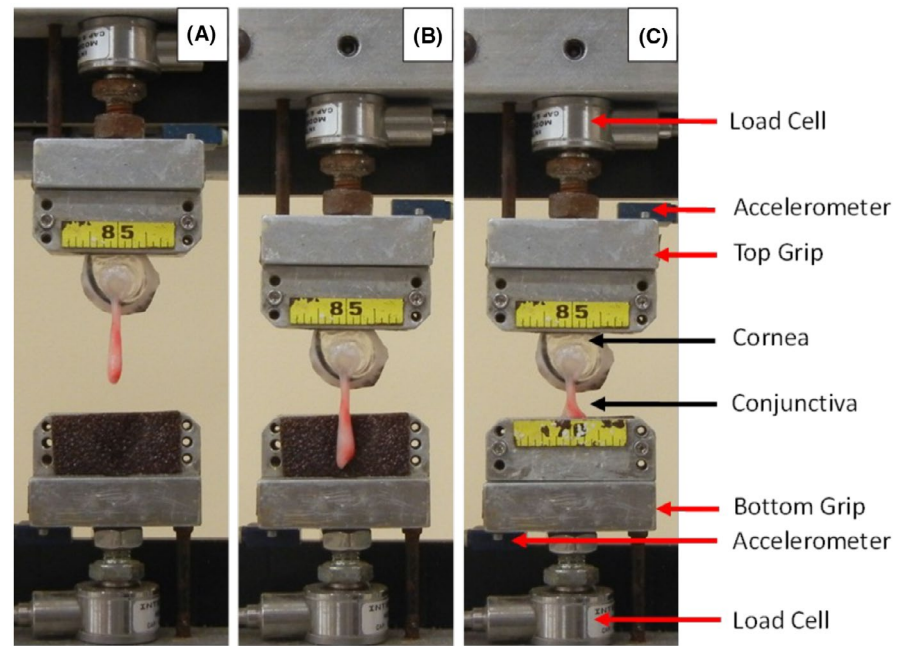


FIGURE 3 Specimen mounting methodology: (A) Top grip assembly and specimen mounted to the testing device with the conjunctival pedicle allowed to hang under its own weight, (B) top grip lowered and specimen placed on the bottom grip, (C) bottom grip clamped onto specimen.



(i.e., 1g of tension) prior to placing the conjunctival pedicle into the bottom grip, inserting approximately 3mm of conjunctival tissue into the grip and then clamping the pedicle (Figure 3). Samples were then quasi-statically loaded to failure at a rate of 1mm/s by moving the top grip while holding the bottom grip stationary (Figure 4). Video was recorded of the front-face of the specimen during each test (Nikon COOLPIX S8100). Data from the load cells, potentiometers, and

accelerometers was acquired at 250Hz (Diversified Technical Systems, TDAS PRO). The data were zeroed by subtracting the respective average pre-trigger data from the entire test. Prior to filtering, all data were truncated at a point just past the peak force and then mirrored and reflected to prevent distortion of the data due to the filter. The mirrored and reflected data were filtered using a 4-pole, phaseless, Butterworth, 20Hz low-pass filter.³⁷ The filtered data from the top and

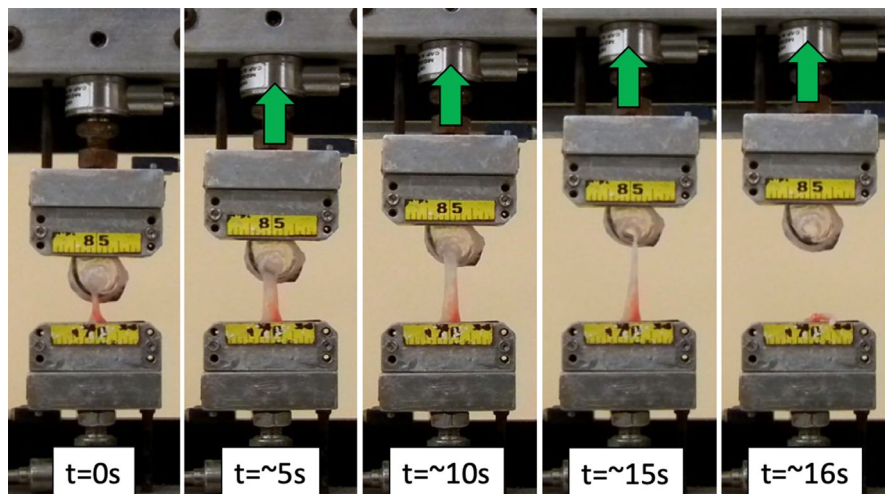


FIGURE 4 Example of specimen being loaded to failure in tension (cyanoacrylate adhesive specimen shown).

bottom load cells were re-truncated to the point just past the peak force, averaged, and then used to determine the peak force for each test.

2.9 | Statistical analysis

Normal probability plots showed that outcomes for average peak load and time to fixation were skewed. Accordingly, data were summarized as median (range). Outcomes were compared between test groups using the Kruskal–Wallis test followed by Dunn's procedure for multiple comparisons. Statistical significance was set to $p < .05$. All analyses were performed using a statistical software package (SAS Institute, SAS Version 9.4).

3 | RESULTS

Forty ex vivo porcine eyes underwent conjunctival pedicle flap surgery, with 6 being omitted for dehiscence prior to tensile testing. Of the 34 tests included in analysis, 10 flaps were secured with suture, 10 with cyanoacrylate, 8 with ReSure[®] hydrogel sealant, and 6 with Tisseel[®] fibrin glue. Peak withstood tensile force results and variance are reported in Table 1. The median(range) measurement for peak withstood force was 0.06 (0.02–0.3), 0.7 (0.44–0.80), 4.4 (1.50–12.10), and 0.1 (0.02–0.18) newtons for ReSure[®] hydrogel sealant, cyanoacrylate glue, sutures, and Tisseel[®] fibrin glue, respectively. A significantly larger peak tensile force was withstood by sutured flap fixation compared to cyanoacrylate glue ($p = .02474$), ReSure[®] hydrogel sealant ($p = .00000$), and Tisseel[®] fibrin glue ($p = .00002$). Cyanoacrylate glue withstood significantly larger peak tensile force than ReSure[®] hydrogel sealant and Tisseel[®] fibrin glue ($p = .01194$ and 0.01798 , respectively). There was no significant difference in peak withstood force

between ReSure[®] hydrogel sealant and Tisseel[®] fibrin glue ($p = .95675$).

The median (range) time to fixation for each group was 25.5 (19–30), 371 (299–953), 770.5 (722–1061), 3601 (3600–3607) seconds for ReSure[®] hydrogel sealant, cyanoacrylate glue, suture, and Tisseel[®] fibrin glue, respectively. Four of the 6 pairwise comparisons for fixation time were statistically significant (Overall p -value $< .0001$). Fixation time for Tisseel[®] fibrin glue was significantly longer than that for cyanoacrylate glue ($p = 0.0016$), and ReSure[®] hydrogel sealant ($p < .0001$). Furthermore, fixation time for sutures was longer than that for ReSure[®] hydrogel sealant ($p = .0003$), and fixation time for cyanoacrylate glue was greater than that for ReSure[®] hydrogel sealant ($p = .0222$). Fixation time for sutures versus Tisseel[®] fibrin glue ($p = .0567$) and for sutures versus cyanoacrylate glue (0.1507) did not differ.

4 | DISCUSSION

To the authors' knowledge, this is the first study investigating the use of adhesives to affix conjunctival pedicle flaps to corneal tissue, including fixation strength comparisons between flaps bonded with adhesives versus conventional suture fixation. Fixation of conjunctival flap to cornea was accomplished by all four methods, but flaps secured with suture fixation were able to withstand approximately 6X the peak applied tensile force when compared to cyanoacrylate adhesive and 70–80X the force compared to ReSure[®] and Tisseel[®] adhesives.

Adhesives have a long history of use in human ophthalmic surgery, dating back to the 1960s in the case of cyanoacrylates. The adhesives used in this study represent the types in regular ophthalmic surgical use today. Cyanoacrylates have been applied in a wide variety of human corneal surgeries, including sealing corneal perforations, managing

Peak force (Newtons)							
Test group	N	Median	Minimum	Maximum	Lower quartile	Upper quartile	Quartile range
Cyanoacrylate	10	0.7	0.44	0.80	0.51	0.73	0.22
ReSure®	8	0.06	0.02	0.33	0.02	0.08	0.06
Suture	10	4.4	1.50	12.10	2.40	7.87	5.47
Tisseel®	6	0.1	0.02	0.18	0.02	0.16	0.14

TABLE 1 Statistical summary of peak tensile forces at point of failure for porcine ex vivo pedicle conjunctival flaps secured to cornea by ophthalmic suture (8-0 Vicryl) and three different adhesive compounds, including ReSure® hydrogel sealant, ethyl cyanoacrylate adhesive, and Tisseel® fibrin glue.

deep ulcers, securing alloplastic grafting materials, limiting fluid egress from cataract surgical incisions, and arresting corneal melts.^{20,38–41} Cyanoacrylates have seen similarly broad application in veterinary ophthalmic practice, including effectively sealing corneal wounds following removal of corneal sequestrum in cats, managing refractory superficial corneal ulcers in dogs and managing perforating corneal injuries.^{42–45} These myriad reports document the efficacy and safety of cyanoacrylate adhesives in corneal application. Several formulations of cyanoacrylates exist, which vary in chemical structure predominantly according to the length of their alkyl side chain. In general, shorter side chain formulations (e.g., methyl and ethyl) are stronger, but exhibit more tissue toxicity than longer alkyl chain formulations (e.g., butyl and octyl) which, while weaker, exhibit greater flexibility. Due to potential tissue toxicity, butyl and octyl formulations are more commonly applied in medical settings, but ethyl cyanoacrylate has been demonstrated to be safe and effective in both human and veterinary corneal surgery (Pumphrey, Kasetsuwan, et al, Barbosa et al). This may be explained in part by the fact that cyanoacrylate histotoxicity is, in part, related to tissue vascularity and the avascular cornea may be less susceptible to such.⁴⁶ Fibrin glues, such as Tisseel®, are not as strong as cyanoacrylate adhesives, but are advantageous in many settings due to their superior biocompatibility. As such, they have been used in a variety of ophthalmic procedures including pterygium surgery, repair of lens capsule injuries, closure of conjunctival incisions, repair of retinal tears, stabilizing keratoplasties and keratoplasties, fixing amnion membrane to the ocular surface and treating deep corneal ulcerations.^{47–56} Ocular PEG-based adhesives are synthetic formulations that currently have a relatively narrow clinical application, but are FDA-approved for ocular use. The predominant ocular use of PEG-based adhesives, such as ReSure®, is in the closure of clear corneal incisions.^{57–60}

While there are no similar studies reported to which we can compare our results, our findings are generally in agreement with Bresnahan et al., who demonstrated that the adhesive strength of cyanoacrylate alone was significantly less than adhesive plus suture material or suture material alone in closure of corneal wounds.⁶¹ Also in agreement with the present study, it has been previously

shown that cyanoacrylate adhesive strength is superior to fibrin glue when affixing porcine tissues (cartilage, bone, and skin).^{62–64} There are several potential explanations for the superior results of cyanoacrylate when compared with Tisseel®. Firstly, it is likely that our results partially reflect cohesive strength, which is the internal strength or the ability of a glue to hold itself together under stress,⁶⁵ as distinguished from adhesive strength, which refers to an adhesive's ability to hold two substrates together.⁴⁴ Additionally, fibrin sealants like Tisseel® require precise and consistent mixing during application. Even with the Duploject® application system, it is possible that the appropriate ratios of sealer protein, fibrinolytic agent, calcium chloride, and thrombin were not met. This was anecdotally reported by Grossman et al. when used for rhytidectomy procedures in humans.⁶⁶ Furthermore, Grossman et al. also reported that any manipulation of the grafted material “after setting” immediately inactivates the clot.⁶⁶ As our study used the highly mobile conjunctiva for grafting material, tissue manipulation during the application, harvesting of the testing tissue bloc, transporting, and tensile testing phases may have lowered the strength of the fibrin adhesive. In the present study, two Tisseel® secured flaps dehiscence prior to transport, despite gentle handling.

In a contrasting report by Shapiro et al., skin wound closure strength achieved by interrupted subcuticular suture was not significantly different when compared to closure by cyanoacrylate.⁶⁷ The authors of that study postulated that the disparate report by Bresnahan et al. was likely due to the difference in chemical structure of the cyanoacrylate products.⁶⁷ Shapiro et al. studied octyl cyanoacrylate while Bresnahan et al. utilized butyl cyanoacrylate.⁶⁷ The physical properties of butyl cyanoacrylate leave it brittle over long distances, whereas octyl cyanoacrylate is a long-chain modification that increases adhesive pliability.^{61,68} It is possible that chemical structure played a role in the results of our study, as the cyanoacrylate side chain in the glue we utilized was an ethyl group, a considerably shorter side chain in comparison to the octyl modification.^{68,69}

Varied chemical structures aside, the mixed findings reported in the literature are not particularly surprising, as drawing comparisons between different tissues, varying testing conditions and different adhesives poses

several challenges. Although consistent tissue types were used across groups within this study (i.e., conjunctiva to cornea), it is reported that tissue condition also plays a role in bonding. Specifically, Chivers and Wolowacz found that the strength of a bond was dependent not only on the nature of the adhesive, but also the condition of tissue being tested.⁶⁴ However, their study showed that the orders of magnitude relative to strength remained constant between adhesives, with cyanoacrylate consistently measuring stronger than fibrin glue.⁶⁴ As most surgeons can attest, tissue condition is difficult to standardize in conjunctival flap surgery under clinical circumstances related to such factors as variation in corneal integrity and conjunctival inflammation. From a surgical perspective, the conjunctiva is not easily stabilized to ensure exacting dimensions during dissection, and consistency of conjunctival flap thickness is also challenging as the underlying connective tissue, Tenon's capsule, may be inconsistently excised, resulting in variable tissue thickness and strength. Considering these sources of variance and to help minimize intergroup variation, a single surgeon was maintained throughout the current study.

For conjunctival pedicle flaps to be successful under clinical conditions, the stability of the corneconjunctival interface provided by the fixation method must last long enough for permanent biologic adhesions to form through the normal wound healing phases of proliferation and maturation. A precise postoperative timeline to achieve permanent pedicle flap adhesion has not been reported but, clinically, donor tissue typically appears to be well integrated into the recipient bed by around 2 weeks post-surgery. Unlike many other locations in the body, as long as the tissues remain coupled, high bond strength may be clinically unnecessary because, although objective data is not available, the tissues are not likely exposed to substantial physical stress. When performed properly, conjunctival flaps should rest in the recipient bed with no appreciable tension, although this may change in the post-operative period due to tissue contraction.^{70,71} Expected *in vivo* sources of stress to the flap include blinking and normal eye movements, neither of which is likely high in magnitude. Additionally, although self-trauma due to rubbing the eye undoubtedly exerts substantially more force to the ocular surface than normal ocular and adnexal movements, utilization of protective measures, such as Elizabethan collars, should substantially reduce or eliminate such impacts on surgical repair sites. With these considerations in mind, it is plausible that cyanoacrylate adhesive, applied in the manner described herein, might provide sufficient fixation strength to the corneconjunctival interface for surgical success. The other two adhesives seem highly unlikely to do so, considering that they resisted significantly less tensile force than cyanoacrylate under the conditions

examined and, in some cases, dehisced prior to tensile testing. Ideally, an objective *in vivo* assessment of the forces to which a conjunctival flap is exposed would help inform this determination. Considering the delicate tissues of the eye, such testing poses significant challenges, but might be accomplished using a method similar to the one used by Lee et al., where stretchable and suturable sensors were used to perform strain measurements on a tendon and ligament in an *in vivo* porcine leg model.⁷²

Although it was not the primary focus of this study, the time to completion of flap fixation was also recorded. The goal of documenting fixation times was to see if one of the adhesive options might potentially achieve a reasonable fixation force, yet exhibit a substantially more rapid fixation time compared to suturing. If so, these combined factors might support it as viable alternative to suturing, which might be particularly useful in the clinical setting for patients possessing comorbidities that preclude prolonged anesthetic times. Use of cyanoacrylate and ReSure® were both significantly faster fixation methods than suturing but, as discussed above, only cyanoacrylate may also possess adequate fixation strength to achieve surgical success as a sutureless alternative.

Several important study limitations must be acknowledged. In addition to a small sample size, this study utilized *ex vivo*, postmortem eyes to test conjunctival pedicle flap fixation integrity. Although adhesives can bond cadaver tissues, it is conceivable that postmortem tissues do not reflect the same integrity or adhesive compatibility as live tissues.^{73,74} Additionally, the forces placed on the *ex vivo* tissues in this study do not necessarily reflect the forces placed on conjunctival pedicle flaps *in vivo*. While sutures are the strongest fixation method, glues may be adequate for typical forces under clinical conditions. In particular, because it withstood substantially more applied force than the other adhesives evaluated in this study, cyanoacrylate adhesive may be capable of maintaining adequate flap fixation to the cornea to allow biologic healing to transpire.

Due to limitations in biocompatibility, the different application approaches used for the different adhesives used in this study resulted in non-standardized adhesive surface areas for the products tested. Both cyanoacrylate adhesive and ReSure® hydrogel sealant are intended to slough off during the healing process and, as such, were placed only over the surface of the conjunctival–corneal interface and extending to the defined perimeter around the keratectomy bed. In this manner these constructs probably relied to a large degree on cohesive strength of the glues, although some of the product likely seeped down the keratectomy wall at the edge of the conjunctival flap, thereby resulting in corneconjunctival adhesion at the flap perimeter. On the other hand, because the fibrin glue is a biologic media and ultimately becomes

incorporated into host tissues, Tisseel® was placed directly in the keratectomy bed under the flap, as well as over the corneal–conjunctival interface of the flap extending to the defined perimeter around the keratectomy bed. While these differences in application are non-standardized from a testing perspective, they accurately reflect limitations of these products in the clinical environment. Lastly, while we utilized a uniaxial testing system in this study, a testing system that utilizes torsional loading, such as a shear rheometer, might be a more ideal, as this approach has demonstrated particular success in measuring tissue bonds of soft adherends (i.e., biologic glues) that are easily damaged and prone to distortion artifact.⁷⁵

In conclusion, the sutureless conjunctival flap fixation alternatives investigated in this study provided significantly less flap security than conventional 8-0 ophthalmic suture. Neither Tisseel® fibrin glue nor ReSure® hydrogel sealant appear to be promising alternatives for this purpose. Further investigation is warranted but, considering its rapid fixation time and ability to withstand significantly greater applied tensile force than the other adhesives studied, ethyl cyanoacrylate adhesive shows some promise as a sutureless option for conjunctival flap fixation.

AUTHOR CONTRIBUTIONS

Elodie VerHulst: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; writing – original draft. **Roxanne M. Rodriguez Galarza:** Conceptualization; funding acquisition; writing – review and editing. **Ian Herring:** Conceptualization; funding acquisition; writing – review and editing. **Andrew Kemper:** Data curation; formal analysis; funding acquisition; investigation; methodology; software; validation; writing – review and editing. **Renata Velloso Ramos:** Writing – review and editing.

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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