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

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Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science
In
Biomedical and Veterinary Science

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January 18th 2023
Blacksburg, Virginia

Keywords: conjunctival pedicle flap, Tisseel®, ReSure®, cyanoacrylate, PEG adhesive, fibrin glue
Comparison of conjunctival pedicle flap to corneal adhesion achieved by Tisseel® fibrin glue, ethyl cyanoacrylate adhesive, ReSure® hydrogel sealant, and conventional suturing with 8-0 VICRYL® suture

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ABSTRACT

**Background:** Conjunctival pedicle flaps are one of the most frequently employed surgical interventions used to address a variety of sight threatening corneal diseases in veterinary ophthalmic practice. 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, suture abscess and dehiscence. In human ophthalmology, a number of sutureless techniques to affix ocular tissues are being explored. Specifically, these approaches include synthetic tissue adhesives, bioadhesives, and hydrogel sealants. The proposed advantages of adhesives over suture, include reduced operative times, watertight seals, decreased foreign-body sensation and inflammation, faster healing times and tissue regeneration with original architecture restoration.

**Objective.** To evaluate the maximum tensile force a conjunctival pedicle flap is able to withstand with respect to different fixation methods, i.e., Tisseel® fibrin glue, ethyl cyanoacrylate adhesive, ReSure® hydrogel sealant, or 8-0 VICRYL suture.

**Animals Studied.** Ex-vivo porcine globes

**Procedures.** Following a 500-micron restricted depth lamellar keratectomy, conjunctival pedicle flaps were dissected and secured to corneal defects with either the bioadhesive Tisseel®, or the synthetic adhesives ReSure®, ethyl cyanoacrylate, or 8-0 VICRYL® suture. Harvested corneoconjunctival flap interfaces were clamped to an accelerometer and potentiometer device, and loaded under video surveillance until the point of failure. Peak load at failure was determined for each test and used to compare between sample types.

**Results.** 40 flaps underwent tensile force testing, with 6 being omitted for dehiscence prior to tensile testing. Of the 34 tests included in analysis, 10 conjunctival flaps were secured with suture, 10 with cyanoacrylate, 8 with ReSure® hydrogel sealant, and 6 with Tisseel® fibrin glue. A significant increase in maximum withstood tensile force was recorded between sutured flap fixation when compared with cyanoacrylate glue (p=0.02474), ReSure® hydrogel sealant (p= 0.00000), and Tisseel® fibrin glue (p= 0.00002). Cyanoacrylate fixation was significantly stronger when compared with ReSure® hydrogel sealant and Tisseel fibrin glue (p=0.01194 and 0.01798 respectively). There was no significant difference in adhesion strength between ReSure® hydrogel sealant and Tisseel® fibrin glue (p=0.95675).

**Conclusions.** Conjunctival pedicle flap fixation using 8-0 VICRYL® suture fixation was able to withstand significantly greater maximum tensile force application in comparison with the ReSure®, Tisseel®, or cyanoacrylate adhesives.
Comparison of conjunctival pedicle flap to corneal adhesion achieved by Tisseel® fibrin glue, ethyl cyanoacrylate adhesive, ReSure® hydrogel sealant, and conventional suturing with 8-0 VICRYL® suture

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GENERAL AUDIENCE ABSTRACT

Conjunctival pedicle flaps are one of the most frequently employed surgical interventions to address sight threatening corneal disorders in companion animals. Due to its redundant nature and close proximity to the corneal surface, conjunctival tissue is readily available for grafting to the cornea. It is surgically dissected to appropriate size and repositioned over the corneal defect where it effectively aids in healing through direct provision of structural support and indirectly via its rich blood supply. Securing the conjunctiva to 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 improper wound he.

In human ophthalmology, a number of sutureless techniques to affix ocular tissues are being explored. Specifically, these approaches include synthetic tissue adhesives, bioadhesives, and hydrogel sealants. The proposed advantages of tissue adhesives over suture, include reduced operation times, watertight closures, decreased foreign-body reaction and inflammatory response, faster healing times and increased ability to induce regeneration of the original tissue architecture.

The purpose of this study was to evaluate the maximum tensile force a corneoconjunctival pedicle flap is able to withstand with respect to four different fixation methods.

40 ex-vivo porcine globes underwent conjunctival pedicle flap procedures. Each pedicle flap was secured to cornea with either 8-0 Vicryl® suture, Tisseel®, ethyl cyanoacrylate, or ReSure®. After harvesting from the globe, the corneoconjunctival unions were clamped to an accelerometer and potentiometer device, and loaded under video surveillance until the point of failure. The peak load was determined for each test and used to compare between sample types.
Acknowledgements

I would like to thank the members of my MS committee, Drs. Roxanne Rodriguez, Ian Herring, and Renata Ramos for their guidance throughout this endeavor.

I would also like to thank Drs. Stephen Werre and Andrew Kemper for their expertise and recommendations; The VVMA Veterinary Memorial Fund for providing funding for this research; the Veterinary Medicine Library at Virginia Tech for providing the 3D printed depth restrictor; and the staff of the Virginia-Maryland Veterinary Teaching Hospital Ophthalmology service, Dr. Steven Hanes, Stephanie Riggins, Terry Wnorowski, and Christa Caldwell-White, for their assistance in materials acquisition and surgery procedure set-up.
Table of Contents

CHAPTER 1: CORNEAL PATHOLOGY LITERATURE REVIEW WITH A PRIMARY FOCUS ON DEEP CORNEAL DEFECTS AND DEEP ULCERATIVE KERATITIS .............................................................................................................1

A. Cornea: Normal Structure and Function .......................................................... 1
B. Corneal Ulcers: Pathogeneses, Clinical Signs and Diagnosis ............................ 3
C. Corneal Defects: Current Treatments and Prognosis ....................................... 6

CHAPTER 2: SURGICAL ADHESIVE REVIEW .................................................... 9

A. Adhesive Properties ............................................................................................ 9
B. Ethyl Cyanoacrylate Adhesive ............................................................................. 10
C. Tisseel® Fibrin Adhesive ....................................................................................... 11
D. ReSure® Hydrogel Adhesive ................................................................................. 14
E. Conclusion and Research Justification ................................................................. 15

CHAPTER 3: COMPARISON OF CONJUNCTIVAL PEDICLE FLAP ADHESION BETWEEN ETHYL CYANOACRYLATE ADHESIVE, TISSEEL® FIBRIN GLUE, AND RESURE® HYDROGEL SEALANT ...................................................... 17

A. Introduction .......................................................................................................... 17
B. Materials and Methods ......................................................................................... 18
C. Statistical Analysis ................................................................................................. 25
D. Results .................................................................................................................... 25
E. Discussion .............................................................................................................. 26

CHAPTER 4: CONCLUSION ................................................................................ 31

REFERENCES .......................................................................................................... 33

APPENDIX: FIGURES ......................................................................................... 43
LIST OF FIGURES

Figure 1: Clotting cascade with fibrin adhesive component.............................43
Figure 2: Representative image of a keratectomy site ....................................44
Figure 3: Conjunctival pedicle flap resting in the keratectomy site .....................44
Figure 4: Duploject® fibrin glue application system ........................................45
Figure 5: Representative image of cornea harvested with a scleral ring ...............45
Figure 6: Harvested conjunctival pedicle flap secured with cyanoacrylate ..........46
Figure 7: Figure 7. Harvested conjunctival pedicle flap (ReSure®) .......................46
Figure 8: Harvested conjunctival pedicle flap (suture) ...................................47
Figure 9: Harvested conjunctival pedicle flap (Tisseel®) ..................................47
Figure 10: Sample Attachments to Accelerometer and Potentiometer ...............48

LIST OF TABLES

Table 1: Summary of peak tensile force statistics ........................................49
Table 2: Summary of fixation time statistics .................................................49
CHAPTER 1: CORNEAL PATHOLOGY WITH A PRIMARY FOCUS ON DEEP ULCERATIVE KERATITIS

A. Cornea: Normal Structure and Function

The cornea is a transparent connective tissue structure that forms the anterior surface of the globe. The positioning of the cornea at the anterior aspect of the globe facilitates vision, but exposes the tissue to the external environment and potential trauma. In order to function for vision and protect the inside of eye from injury, the cornea relies on a structure that balances inherent mechanical strength with transparency.¹

Broadly, the cornea is comprised of three distinct layers: an outermost epithelial layer, middle stromal layer, and an innermost monolayer of endothelial cells. Across species, these cell layers combined generally remain less than one millimeter thick.²⁻⁶ Ultrastructurally, the cornea is comprised of an intricate network of keratocytes, nerve fibers, water and extracellular matrix. Under normal anatomic and physiologic conditions, this composition and morphology results in a clear curved window allowing for transmission of light through to the visual axis. The cornea focuses light onto the lens and retina, a process wherein maintenance of clarity is paramount to vision.

Corneal clarity is multifactorial and achieved through several anatomic characteristics. The blood supply to the eye is provided by the ophthalmic artery which branches extensively to serve almost all ocular tissues, stopping just short of entering the cornea. Because blood vessels are not translucent, maintenance of corneal avascularity is one key to its transparency. Lacking blood vessels, the cornea is nourished through aqueous humor and the pre-corneal tear film, both of which are transparent in the normal state.⁷ On a subgross level, corneal clarity is further facilitated by the absence of opaque cellular products like the protein keratin and pigment, such as melanin. Because the
biologic activity of melanin is specifically intended dissipate light, it is logical that its presence in the visual path would be counterproductive to vision, which requires the uninhibited transmission of light.8 Keratin is another cellular product that is found in abundance in the body (e.g. cutaneous epithelium), but it absent in corneal epithelium. Similarly to melanin, the presence of keratin within the corneal epithelium would increase light scatter and be counterproductive to vision. In diseased states, both melanin and keratin have increased precence in the cornea and can significantly impact its overall optical capacity.9,10 Some corneal proteins are actually thought to facilitate light transmission. Specifically, it has been shown that corneal cells express water soluble proteins colloquially termed “corneal crystallins.”11,12 These proteins are reminiscent of the lens protein crystallins that give transparency and refractive power to the lens.11-13

Beyond the individual cellular features, it is widely accepted that the precise arrangement of the cells with their surrounding extracellular matrix is a major contributor to corneal clarity. In the cornea, extracellular matrix is composed of water, collagen, glycosaminoglycans, and proteoglycans. Types of collagens found in the tissue include I, III-VIII, and XII with type I being the most common.14-18 Ultrastructurally, the individual corneal collagen fibrils are D-periodic (linear axial growth assembly).19 The D-periodicity of the fibril refers to the side-to-side associations of triple-helical collagen molecules. The D-stagger of collagen molecules produces a regularly recurrent pattern.19,20 These highly patterned collagen molecules bind together covalently to form fibril bundles which build further into an exact lamellar configuration. The individual fibrils The assembly is a thin, intricate network of fibers aligning into a precise latticework that reduces diffraction. 21,22 The network is further modulated by core
proteins associated with proteoglycans that limit lateral interaction of growing lamellae.

The end result of this construction is a perfectly clear, curved optic that transmits light at the start of the visual pathway.

A final factor in corneal clarity is a strict water balance. Water regulation is imperative as the cornea is constantly bathed in fluid; that is aqueous humor on the anterior chamber side and the pre-corneal tear film on the ocular surface. The pre-corneal tear film contributes to clarity by smoothing out ocular surface microirregularities and forming a unified interface between air, tears and cornea, which is responsible for two thirds the total refractive power of the eye. Despite this constant contact with water, the cornea itself is maintained in a constant state of dehydration. At the anterior surface of the cornea, influx of aqueous tears is physically inhibited through epithelial anatomic barriers. Deturgescence on the endothelial side is an active process, achieved by channeling water out of the stroma via Na+/K+ ATPase pumps and through osmotic gradients. Specifically, sodium and calcium ions pass into the aqueous humor via an energy consuming pump action and corneal stromal water passively follows. When corneal disease results in dysregulation of the fluid dynamics, corneal swelling or edema results. The edema disrupts the highly ordered arrangement of the lamellae and ultimately diffracts light. Clinically this appears an opaque, blue hued, cornea.

B. Corneal Ulcers: Pathogenesis, Clinical Signs, and Diagnosis

The exposure of the cornea to the external environment makes it susceptible to injury. However, several innate mechanisms exist to help protect the globe and limit instances of corneal trauma. Briefly, the cornea is highly innervated by the long ciliary nerves which extend from the ophthalmic branch of the trigeminal nerve.
(afferent) input via these nerves ultimately leads to the protective efferent actions of blinking the eyelids (cranial nerve VII) and retraction of the globe (cranial nerve VI) to protect the eye. This neurologic sequence is the basis for the corneal and palpebral reflexes. In addition to closure of the eyelids, many animals have a third eyelid which can either actively or passively elevate to serve as physical barrier between the cornea and a noxious stimulus. Animals also take an active role in protecting their corneas through learned behaviors such as the menace response in which they recognize a threatening movement toward the eye and immediately blink and retract the globe. Lastly, but not insignificantly, the pre-corneal tear film should be recognized for its role in maintaining and protecting a healthy ocular surface. Tears are a cohesive phase comprised of lipid, aqueous and mucin that act collectively to nourish the avascular cornea, prevent desiccation, and remove waste and debris as part of their barrier role.

Despite all of these defense mechanisms, failures leading to corneal injury, including ulceration, do occur. The diagnosis of ulcerative keratitis is broad, but always presumes a complete (focal or regional) loss of the outer epithelial cell layer exposing and sometimes involving the underlying stroma. When the corneal defect extends into the stroma, ophthalmologists further qualify the disease process as “deep” or “complicated” corneal ulceration. In veterinary ophthalmic practice, both superficial and complicated corneal ulceration are common. Corneal ulceration can occur spontaneously, but is more commonly the result of causes such as congenital defects, trauma, foreign bodies, infections, pre-corneal tear film deficiencies, degenerative keratitis, eyelid abnormalities (entropion, ectropion, euryblepharon), and eyelash disorders (distichiasis, ectopic cilia, trichiasis), among others. Additionally, surgical removal of corneal
tissue is often necessitated to address corneal diseases such as neoplasia, abscessation and corneal sequestration, among others. Regardless of cause, corneal wounds are similar in consequence, including risk of perforating keratitis, potential loss of the globe and subsequent visual impairment.

Because the cornea relies on clarity for its visual function, any breakdown in its architecture impairs its ability to allow unimpeded light transmission. Even a healed corneal injury may result in permanent scar tissue, which may negatively impact vision. For these reasons, ophthalmic medical and surgical interventions are not only aimed at preserving the globe, but also at limiting tissue destruction and scar tissue formation, in order to preserve the highest quality vision possible.

Clinical Signs & Diagnosis

Presenting signs of ophthalmic disease, in general, may be non-specific and corneal disease is no exception. Clinical observations may include signs of discomfort as demonstrated by blepharospasm, photophobia, and epiphora, or may be more pointed changes like mucopurulent discharge, conjunctival hyperemia, corneal edema, neovascularization, infectious infiltrate, gross to sub-gross corneal surface irregularities and varying degrees of reflex uveitis (intraocular inflammation).

A specific diagnosis of *deep* corneal ulceration is typically made via gross evaluation of the cornea, plus slit-lamp biomicroscopy and fluorescein staining. Slit-lamp biomicroscopy allows magnified evaluation of adnexa, cornea, and anterior chamber. It also helps to illuminate sub-gross corneal changes which in turn, aids in determining prognosis and appropriate course of therapy. Additionally, slit-lamp biomicroscopy allows visualization of a cross-sectional image of the cornea, providing specific insight
into depth of corneal wounds.\textsuperscript{29,33} Having a quasi-accurate way to determine depth bears significant impact on prognosis and treatment decisions. Fluorescein staining is often the final step in corneal ulcer diagnosis and provides an objective parameter, dying only exposed stroma through hydrophilic affiliation.

\section*{C. Corneal Defects: Current Treatments}

Once a diagnosis of corneal ulceration is made, appropriate treatments are typically dictated by underlying disease, presence of infection and, importantly, how fragile the remaining cornea is. In domestic animals, normal corneal thickness is generally less than one millimeter.\textsuperscript{2,6,34} Due to this inherent thinness, injuries or ulcerations that result in loss of more than half of the corneal thickness (as determined by slit lamp biomicroscopy) often require surgical intervention to help ensure uncomplicated healing and preservation of a visual and comfortable eye.\textsuperscript{25,35} When deciding what treatment option is best, clinicians must often compromise on a procedure that maximizes the odds of saving the eye, yet minimizes disruptions to clarity.

Surgical techniques to provide tectonic support are varied, but typically utilize a support material which may be autologous, homologous, or biosynthetic.\textsuperscript{36-45} For the purposes of this project and due to the frequency at which they are performed in veterinary ophthalmic practice, we will focus on an autologous-type surgical procedure called conjunctival pedicle flapping.

Application of a conjunctival flap to the corneal surface is one of the most frequently employed veterinary surgical interventions used in restoration of integrity to a fragile cornea and is applicable to many companion animal species.\textsuperscript{38,46,47} Due to its redundant nature and close proximity to the corneal surface, conjunctival tissue is readily
available for grafting to the cornea. It is surgically dissected to appropriate size and repositioned over the corneal defect and secured there, where it effectively aids in healing through direct provision of structural support and indirectly via its rich blood supply. Described conjunctival flapping and grafting techniques include the conjunctival pedicle flap, conjunctival hood flap, conjunctival island graft, and 360-degree conjunctival flap.\textsuperscript{30,37-39,46-50} The specific technique employed is based upon the clinical scenario and surgeon preference. However, in veterinary medicine, conjunctival pedicle flaps are most commonly employed because they can be used for lesions located anywhere on the cornea and can result in minimal visual impairment and an intact vascular supply.\textsuperscript{30} Because conjunctival flaps and grafts are autologous, rejection and transmission of disease are unlikely. Conventionally, conjunctival flaps are anchored over the corneal defect by suturing, which is necessary to maintain adhesion in the early postoperative period. In the days and weeks following surgery, more permanent adhesion occurs through vascular ingrowth from the conjunctiva and connective tissue bridging between the cornea and conjunctiva.

Due to their delicate nature, suturing corneal and conjunctival tissues is technically challenging, requiring a skilled surgeon and operating microscope. Surgical times can be prolonged and the end results are not without complication. The success of a flap is measured by its ability to integrate with the cornea during the healing process, thereby maintaining a functionally visual and comfortable eye. A 2001 human retrospective study reported up to 34\% of cases experienced suture-related complications post keratoplasty.\textsuperscript{51} Conjunctival flap procedures have proved reliable with success rates anywhere from 90-100\%.\textsuperscript{38,46,48,52,53} Some of the complications associated with sutured
conjunctival flaps include, infection, suture reaction, scarring and dehiscence with the rate of dehiscence reported as 4-7%.\textsuperscript{51,53} In order to minimize inflammation associated with absorbable suture breakdown (by hydrolysis reaction) following corneal surgeries in humans, non-absorbable sutures are used almost exclusively. However, removal of non-absorbable sutures requires an additional surgery, which poses a limitation in veterinary patients. Due to the practical need to utilize absorbable suture material in veterinary patients (i.e. to limit additional surgery costs and anesthesia), the prolonged inflammatory reaction to the presence of suture material in the cornea is an accepted trade-off, which can result in debilitating visual consequences, including corneal vascularization, edema, scarring and pigmented keratitis.\textsuperscript{51,54} In addition, astigmatism (of a >5 diopter difference) following keratoplasty suturing in humans has been reported in up to 17-20% of cases.\textsuperscript{55,56} This often necessitates additional treatment such as corrective lenses, re-performing corneal grafting or Laser in situ keratomileusis (LASIK) to aid in improving visual acuity. Given the well-established risks of suture related complications, clinician scientists have moved to explore sutureless wound closure techniques.
A. Adhesive Properties and Classifications

Adhesive materials have been advocated as an alternative to suture in both human and veterinary surgical practice. Adhesives have wide-ranging clinical applications that are founded on some basic principles including their ability to bond tissues, fill potential space, reduce or eliminate bleeding, support healing, create a protective wound barrier, and regulate wound moisture content. In general, the requirements of adhesives can be subcategorized into chemical, physical, and biological properties. The evolving role of adhesives in niche sub-specialty procedures exploits these different properties.

It is the chemical properties of adhesives that allow for bonding to occur. To facilitate bonding, adhesives need to be chemically transformed from a liquid to solid state. This can occur through polymerization, chemical crosslinking or the evaporation of solvents. Polymerization is a chemical reaction combining small molecules (monomers) into larger repeated structured units (polymers). Many of the modern medical adhesives, namely cyanoacrylates are cured through polymerization. Similarly, chemical crosslinking also adjoins molecules, specifically through the formation of covalent bonds using a chemical reagent. In short, the covalent bonding occurs because two or more atoms have an electrostatic attraction for the same electron(s). So. This process works when polymers exist in a semi-liquid state, but solidify to bond further through the evaporation of a chemical solvent. Regardless of how adhesives are cured, it is the resultant physical properties that dictate their specific uses in medicine and surgery. Ideally, the final material will mimic the mechanics of normal neighboring tissues. For the purposes of achieving corneal adhesion and wound healing, this generally requires
limited swelling or shrinking of the adhesive with provision of sufficient tensile strength to withstand the natural forces on the eye. Although numerous adhesive materials exist, the scope of this thesis will restricted to cyanoacrylate, fibrin, and hydrogel sealants.

B. Ethyl Cyanoacrylate Adhesive

Cyanoacrylate based synthetic adhesives have been used in in human ocular surgery for roughly sixty years, with some of the first applications being in corneal surgery.\textsuperscript{59-62} In 1962 Bloomfield et al. demonstrated several variations in adhesion including conjunctiva to conjunctiva, adhesive to cornea, and conjunctival flap to sclera.\textsuperscript{59} In the years since, cyanoacrylates have seen continued use in human corneal surgery in sealing corneal perforations, patching deep ulcerations, securing alloplastic grafting materials, limiting fluid egress from cataract surgical incisions, and arresting corneal melts.\textsuperscript{60,63-67} In the veterinary medical literature, cyanoacrylate adhesives have been used similarly. Pumphrey et al. demonstrated cyanoacrylate effectively patching corneal wounds after a subtotal removal of corneal sequestra in cats.\textsuperscript{68} In dogs, refractory superficial ulcerations have been treated successfully with the application of cyanoacrylate glue.\textsuperscript{69} Even deep to perforating corneal injuries have been managed in veterinary patients using cyanoacrylate.\textsuperscript{40,70}

The advantages to cyanoacrylates are many. The material itself is well tolerated, inciting only mild local inflammation.\textsuperscript{71} The bonding process is rapid and is achieved when monomeric esters polymerize through anionic activity initiated by water or a weak base.\textsuperscript{69} The strength of cyanoacrylate bonds is arguably one of their most desirable qualities. In one study by Banitt et al., cyanoacrylate was able to withstand the highest intraocular pressure when compared with suture and fibrin glue after closure of clear
Corneal incisions.\textsuperscript{72} Cyanoacrylates have also demonstrated superior wound closure strength in skin when compared with suture material.\textsuperscript{73} Cyanoacrylates not only bond tissue, but they have other desirable biologic properties, including antimicrobial activity and anti-proteolytic properties.\textsuperscript{74-76} In 1983, Eiferman et al. reported antimicrobial activity of cyanoacrylate against gram positive organisms.\textsuperscript{76} Furthermore, cyanoacrylates do not interrupt the corneal healing process.\textsuperscript{71}

The application of cyanoacrylates on the cornea is not without complication. During the polymerization process, localized heat causes cellular toxicity.\textsuperscript{77} It is also speculated that if the corneal wound is down to Descemet’s membrane, the heat generated may be enough to rupture the eye. Other toxicities occur during the degradation process in which formaldehyde is released furthering local cell death.\textsuperscript{78} However, as with many toxins, the tolerance to cyanoacrylate adhesives has been determined to be dose dependent. The amount of glue used impacts the amount of inflammation.\textsuperscript{60,71} In the context of ocular surgery, the volume of glue is relatively small and proven to be well tolerated.\textsuperscript{71} With scientific advances and refinement of cyanoacrylate adhesives, some of their undesirable properties have been dampened by modifications to their chemical structure (e.g. ethyl cyanoacrylate vs N-butyl cyanoacrylate). Ultimately, discretion during case selection is important, but cyanoacrylates remain a viable option for corneal wounds and may serve as an alternative to suturing for fixation of conjunctival pedicle flaps to cornea.

C. \textit{Tisseel}\textsuperscript{®} Fibrin Adhesive

Biologic adhesive materials derived from human blood, such as fibrin glues, have also emerged in medical use, with a unique set of advantages when compared with synthetic adhesives. In contrast to cyanoacrylate-based glues, fibrin glues are non-toxic,
promote healing and are biodegradeable.\textsuperscript{79-83} As early as 1944 fibrin was used to help fix skin grafts to wounded soldiers.\textsuperscript{84} In the 1970s fibrin glues were used to reunite peripheral nerves in an animal model by Matras et al.\textsuperscript{85} The same team reported successful use of fibrin glue in human applications by 1973.\textsuperscript{86} Presently, the blood derivative products have evolved into a highly concentrated cryoprecipitate as a basis for two-component sealants, like the commercially available Tisseel\textsuperscript{®}. The mechanism by which Tisseel\textsuperscript{®} bonds tissues is through mimicry of the final steps in coagulation cascade. Briefly, fibrinogen must be converted into fibrin monomers by the addition of thrombin. The fibrin monomers then polymerize into a stable clot when combined with cofactors calcium chloride and factor XIII (see Figure 1 below). Normally, the coagulation cascade continues in the body by undergoing a degradation process in which plasmin breaks down the clot. However, one of the added components of commercial fibrin glues is aprotinin, which inhibits the transition of plasminogen to plasmin prolonging the life and integrity of the clot, thus prolonging the desired adhesion time.\textsuperscript{87,88}

The biologic benefits of fibrin adhesives are many. Although not bactericidal like cyanoacrylates, fibrin has demonstrated some anti-infectious properties with staphylococcus aureus growing less in the fibrin clot.\textsuperscript{89} In comparison to suture material, fibrin glues have been reported to be less painful when used in ophthalmic practice, specifically.\textsuperscript{79} Perhaps the most advantageous feature of fibrin glues, is their ability to facilitate wound healing. Fibrin adhesives contain concentrated transforming growth factor β (TGF-β) and vascular endothelial growth factor (VEGF). These are believed to promote tissue reconstruction by stimulating the proliferation of fibroblast cells and angiogenesis, respectively.\textsuperscript{90,91} In human medicine, autologous fibrin glues have
significantly accelerated epithelialization of surgically created wounds. In veterinary practice, fibrin glues have been used for anastomoses and skin closures. Fibrin glues have been used in a variety of ophthalmic procedures including to repairing perforating injuries to lens capsules, closing conjunctival incisions, repairing retinal tears, stabilizing keratoprosthesis, treating deep to penetrating keratoplasties, fixing amnionic membrane to the ocular surface, and treating deep corneal ulcerations. As part of the justification for fibrin glue use in this study, we extrapolated the results from Foroutan et al. where there was successful adhesion of conjunctiva to corneal tissue.

Despite the aforementioned advantages, there are pitfalls in using human biologic products as adhesives. A primary concern with commercially available fibrin glues in human application is disease transmission. Historically, viruses like human immunodeficiency virus (HIV) and hepatitis have both been implicated in donated blood product disease transmission. This can be combatted through viral inactivation techniques of allogenic blood or strictly using an autologous donation. Aside from clinic personnel handling/preparation, the concern of disease transmission from a human biologic to patients may be assuaged in veterinary medicine as it is widely accepted that the viruses in question are unlikely to cross species and infect our animal patients.

Disease transmission is of no consequence for our purposes in this study, due to the ex-vivo experimental design. Perhaps the largest pitfall for veterinary clientele is the cost of commercially available fibrin products. The product used in this study (Tisseel®) is roughly ten times the cost of suture material and likely cost prohibitive in the clinical setting, unless significant clinical advantages are identified which might outweigh the financial cost of the product.
D. ReSure® Hydrogel Sealant:

In another attempt to overcome some of the challenges posed by suture and cyanoacrylate glues, an adhesive formulation from polyethylene glycol (PEG) has been developed. PEG adhesives are biocompatible and possess many of the desired characteristics of an ideal medical glue. Like most adhesive products, PEG-based glues are cured through polymerization. In essence, a PEG compound with a specific ester or carbonate end group is combined with another polymer that contains amine or thiol nucleophiles. The end result is a hydrogel polymer affixed by amide or carbamate linkages.111

Among the commercially available polyethylene glycol products, several have been specifically designated for use in ophthalmic practice. In one human cataract study, OcuSeal®, which is marketed as a liquid bandage, achieved integrity of clear corneal incisions up to an intraocular pressure of 246 mmHg before fluid egress.112 Another study found that when compared to stromal hydration, OcuSeal® resulted in superior wound closure of clear corneal incisions post cataract surgery.113 The same study by Uy also demonstrated that when compared to traditional suturing, OcuSeal® was better tolerated with less foreign body sensation and less astigmatism.113 The ocular PEG adhesive, ReSure® was specifically chosen for this study because it showed similar results to OcuSeal® in that it was effective in closing clear corneal incisions with the benefit of being readily available and FDA approved.113-116 ReSure® carries with it several other desirable properties including ease of application ~99.7% of time.117,118 ReSure® is concealed, smooth and sloughs completely during re-epithelialization.115,116,119 When
specifically compared to conventional suture material, ReSure® was shown to decrease suture related complications including mitigating wound leakage, lowering incidence of subconjunctival hemorrhage, and decreasing foreign body sensation. The reported complications of ReSure® are few. The main downfalls are that the glue cures too rapidly to allow for sufficient application in some cases and relies on a relatively dry application bed.

E. Conclusion & Research Justification

When considering adhesives as tissue coupling agents, researchers have been tasked with finding products that can maintain adhesion under the natural forces impacting native tissue. When it comes to bonding conjunctiva to cornea, there should be no tension on the flap when it is affixed to the corneal bed. However, once a patient is awakened from surgery, natural movements of the globe and movement of eyelids over the ocular surface likely causes some amount of tension. Although presumably small, the magnitude of this force remains unreported. Demonstration of any of the above adhesives to effectively and efficiently bond conjunctiva to cornea during conjunctival pedicle flap surgery might bring significant advantages to this common procedure in veterinary ophthalmic practice.

This study investigated the efficacy of tissue bonding by adhesives in conjunctival flap surgery by comparing the tensile strength of four different techniques, including suture and three adhesives, in securing conjunctival pedicle flaps to corneal wounds. We hypothesized that suture material would provide higher tensile strength than adhesives. Additionally, we expected lower surgical times in the adhesive groups when compared
with the suture group. If successful adhesions were achieved and tension tolerance was similar across groups, this would support sutureless options being investigated further as a viable technique for securing conjunctival pedicle flaps to cornea in the treatment of deep ulcerative keratitis and other conditions requiring this surgical procedure.
CHAPTER 3: COMPARISON OF CONJUNTIVAL PEDICLE FLAP TO CORNEAL ADHESION ACHIEVED BY ETHYL CYANOACRYLATE ADHESIVE, TISSEEL® FIBRIN GLUE, RESURE® HYDROGEL SEALANT, AND OPHTHALMIC SUTURE

A. Introduction

Ulcerative keratitis is a common ocular surface disease with a prevalence of approximately 0.8% in the general canine population.121 With appropriate treatment, most 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 sequela. Once the cornea has reached a fragile state, surgical interventions to provide tectonic support may become necessary. Conjunctival flaps and grafts are the most frequently employed surgical procedures performed to address deep corneal defects in veterinary ophthalmic practice.25,38,46 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.7,25,30,37-39,46,48,50,53,122 Of the described surgical techniques, all exploit the same set of 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 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. In human ophthalmology, a number of sutureless techniques to affix ocular tissues are utilized or are being explored. Among others, these approaches include the use of synthetic tissue adhesives, bioadhesives, and hydrogel sealants. The proposed advantages of tissue 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.

In this study we performed keratectomy and conjunctival pedicle flap surgery on ex-vivo porcine globes utilizing four discrete security techniques including suture, cyanoacrylate, ReSure® hydrogel sealant, and Tisseel® fibrin glue. The aim of the project was to determine and compare the tensile strength of the surgical fixation of conjunctival flaps to cornea achieved by these four methods.

B. Materials and Methods

Ocular Tissue Samples

Ex-vivo porcine globes were obtained (Animal Technologies Tyler, Texas US) within 24 hours 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 corneoconjunctival abnormalities not attributable to expected post-mortem findings were excluded from the study.
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, California, United States) was selected as the biologic adhesive. Ethyl cyanoacrylate adhesive (All-purpose Krazy Glue®; Elmers Products, High Point, NC) represented the synthetic glues. ReSure® hydrogel sealant (Ocular Therapeutix, Massachusetts United States) was used as a polyethylene glycol (PEG)-type adhesive and VICRYL® 8-0 (Polyglactin 910 with 5.5mm 1/2c spatula, MWI Animal Health, Idaho, United States) as the suture fixation.

Study Design

Four treatment groups (A-D), each utilizing 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 four non-consecutive days. Experimental procedures for each treatment group were performed in their entirety on each of the four study days. Groups A through D were experimental days 1 through 4 respectively. On the day of arrival, eyes were selected via random drawing. Once examined and deemed free of corneoconjunctival 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 table-top operating stage. Surgical loupes with 3.5x magnification were used throughout the surgical procedures.

*Conjunctival Pedicle Flap Preparation*

For each specimen, the conjunctival pedicle flap was dissected first. Starting at the 9 o’clock position approximately 1 millimeter posterior to the limbus, the donor bulbar conjunctiva was separated from the globe and Tenon’s with a combination of blunt and sharp dissection. The tissue was harvested 90 degrees in the dorsal temporal direction and the base was left intact at the 12 o’clock position. The flap was approximately 7mm wide. Care was taken to undermine and free the conjunctiva tissue from Tenon’s capsule. If a conjunctival “button hole” occurred, the globe was discarded and replaced with a new specimen.

*Keratectomy Procedure*

After conjunctival flap dissection was complete, a 6mm corneal stromal defect serving as the flap recipient site was made via keratectomy. In order to improve tissue sectility during keratectomy, globes were reinflated via intracameral injection at the ventral (6 o’clock) position using water and a 3mL syringe with 30gauge needle. Focal stromal hydration was employed to prevent leaking at the injection site. Briefly, a 6 mm diameter punch biopsy outfitted with a 3D printed depth restrictor was used to create a 500 micrometer deep corneal defect centered three 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 (see Figure 2 below). To ensure a consistent surface contact area for the adhesives, a one-millimeter
rim of epithelium was removed around the perimeter of the keratectomy site. This defined perimeter was accomplished using Castroviejo calipers for alignment of an 8mm punch biopsy surrounding the recipient site. The one-millimeter rim of epithelium was lifted with a 6400-beaver blade and peeled or sharply excised around the recipient site. The region served as a delineated moat to prevent adhesive from migrating past this defined point and to ensure that the applied adhesive surface area was uniform between samples.

Once the keratectomy bed was prepared, the conjunctival pedicle flap was rotated ventrally until resting within the surgical defect without tension (see Figure 3 below). When necessary, the flaps were trimmed to size to reside within and flush with the 6mm recipient bed border.

Conjunctival Pedicle Flap Fixation

A timer was used to record the amount of time, in seconds, required for completion of conjunctival pedicle flap fixation to the cornea, to include the manufacturer-recommended polymerization or clotting time of the sealant material or the completion of suturing.

Group A (ethyl cyanoacrylate): The corneal and conjunctival surfaces were dried until tacky with Weck-Cel® spear (Intervet Inc. Merck Animal Health, Summit, NJ). The conjunctival edges were checked to ensure they were situated within and abutting the edges of the 6mm recipient bed. Ethyl cyanoacrylate adhesive was manually applied with a fine-tip paint brush over the corneconjunctival 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®): Similarly to group A, the corneal and conjunctival surfaces were dried until tacky with Weck-Cel® spear (Intervet Inc. Merck Animal Health, Summit, NJ). The conjunctival edges were checked to ensure they were situated to reside within and flush with the 6mm recipient bed border. ReSure® poly ethylene 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 one-millimeter 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. Single 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, after suture was passed through the conjunctival flap, corneal sutures consistently entered the cornea at the deepest aspect of the recipient bed.

Group D (Tisseel®): As with groups A and B, the corneal and conjunctival surfaces were dried until tacky with Weck-Cel® spear. The pedicle flap conjunctival edges were checked to ensure they were situated to reside within and flush with the 6mm recipient bed border.
The fibrin glue was then 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 exact 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® application system provided (see Figure 4 below). The glue did not extend past the one-millimeter delineated moat. The cornea/flap/adhesive interface was allowed to clot until solidified.

Harvesting the cornea:

Following conjunctival flap fixation, the pedicle flap base was freed by sharp transection from the bulbar aspect of the globe. The entire cornea including the conjunctival flap interface was sharply excised with an attached 1-2-mm wide scleral border and intact pedicle flap (see Figures 5-9 below). The tissue samples were transported to the Virginia Tech College of Engineering, Impact Biomechanics Laboratory in a humidity-controlled container for tensile testing.

Tension 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, Irwin, PA), which were operated with a multi-axis controller (Parker, ACR9000, Irwin, PA) and motor driver (Parker, ViX, Irwin, PA). Each of the stages was instrumented with a single-axis load cell (Interface, WMC-5, Scottsdale, AZ), accelerometer (Endevco, 7264B-2000G, Irvine, CA), and potentiometer (Firstmark Controls, Creedmoor, NC). A previously developed testing procedure was used to maintain consistency in initial specimen slack and alignment. 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, i.e., 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 prior to clamping the conjunctival pedicle into the bottom grip (see Figure 10 below). Samples were then quasi-statically loaded to failure at a rate of 1 mm/s by moving the top grip while holding the bottom grip stationary. Video was recorded of the front-face of the specimen during each test (Nikon COOLPIX S8100, Melville, NY). Data from the load cells, potentiometers, and accelerometers was acquired at 250 Hz (Diversified Technical Systems, TDAS PRO, Seal Beach, CA). 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, phase less, Butterworth, 20 Hz low-pass filter. The filtered data from the top and 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. The average, median, and
standard deviation for peak force were calculated for each test group/sample type and used to compare between test groups/sample types.

C. 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<0.05. All analyses were performed using a statistical software package (SAS Institute, SAS Version 9.4, Cary, NC).

D. Results

Peak tensile force results are reported in Figure 11. 40 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. The median(range) measurement for peak force was 0.1 (0.02-0.33), 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 average peak tensile force was withstood for sutured flap fixation compared to cyanoacrylate glue (P=0.02474), ReSure® hydrogel sealant (P= 0.00000), and Tisseel® fibrin glue (P=0.00002). Cyanoacrylate glue withstood significantly larger average peak tensile force than ReSure® hydrogel sealant and Tisseel® fibrin glue (P=0.01194 and 0.01798,
respectively). There was no significant difference in peak force between the bioadhesives ReSure® hydrogel sealant and Tisseel® fibrin glue (P=0.95675).

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. All fixation time comparisons were statistically significant with Tisseel® fibrin glue being a significantly longer fixation time than all others, followed by suture, cyanoacrylate glue, and ReSure® hydrogel sealant, respectively. The fixation times are summarized below in Figure 12.

E. Discussion

To the author’s knowledge this is the first study investigating the adhesion strength that different classes of medical adhesives and conventional suture material are able to achieve in binding conjunctival pedicle flaps to cornea, as measured by the peak tensile force the conjunctival flap adhesions achieved by these fixation methods are able to withstand. Adhesions were achieved by all four tested materials, but the suture fixation method was able to withstand 70-80X the peak applied tensile force when compared to the PEG adhesive ReSure® and the fibrin adhesive Tisseel®, and 6X the force when compared with cyanoacrylate glue. These findings were in agreement with Bresnahan et al. who demonstrated that the adhesive strength of cyanoacrylate-based adhesive was significantly less when compared to adhesive plus suture material or suture material alone in closure of corneal wounds. 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). There are several potential
explanations for the superior adhesion strength of cyanoacrylate when compared with Tisseel®. Firstly, it is possible that that our results were impacted by cohesive strength, which is the internal strength or the ability for a glue to hold itself together under stress.\textsuperscript{141} This differs from adhesive strength, which refers to an adhesive’s ability to hold two substrates together\textsuperscript{70}. Even with video surveillance, the physical breakpoint was not always obvious for fibrin glues. Fibrin glue is clear and colorless, so whether the glue itself broke apart from poor cohesive strengths that was interpreted as poor adhesive strength is unknown. Second, 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.\textsuperscript{142} Furthermore, Grossman et al. also reported that any manipulation of the grafted material “after setting” immediately inactivates the clot.\textsuperscript{142} 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 initial adhesion strength or potentially the cohesive strength. In the present study, two Tisseel® secured flaps dehisced prior to transport, despite gentle handling.

In a contrasting report by Shapiro et al., wound closure strength achieved by interrupted subcuticular suture was not statistically different when compared to closure by cyanoacrylate.\textsuperscript{73} 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. Shapiro et al. studied octyl cyanoacrylate while Bresnahan et al.
utilized butyl cyanoacrylate.\textsuperscript{73} The physical properties of butyl cyanoacrylate leave it brittle over long distances, whereas octyl cyanoacrylate is a long chain modification that provides increased pliability and strength.\textsuperscript{138,143} It is possible that chemical structure played a role in the results of this study as the cyanoacrylate side chain in the material utilized was an ethyl group, a considerably shorter side chain in comparison to the octyl modification.\textsuperscript{143,144}

Even without varied chemical structures, the mixed findings reported in the literature are not particularly surprising, as drawing comparisons between different tissue testing conditions and different adhesives poses many challenges. Across the referenced literature, numerous different tissues types have been utilized. Although the same tissue types were used across groups within this study (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 largely dependent on the nature of the adhesive and the condition of tissue being tested.\textsuperscript{140} Although bond strengths varied in the different tissue tests, their study showed that the orders of magnitude remained constant between adhesives with cyanoacrylate consistently measuring stronger than fibrin.\textsuperscript{140} As most surgeons can attest, tissue condition is hard to standardize in conjunctival flap surgery under clinical circumstances. This may be related to variation in corneal integrity and conjunctival inflammation. The flexible conjunctiva is not easily stabilized to ensure exact dimensions during dissection. Consistency of conjunctival flap thickness is also challenging as the underlying connective tissue, Tenon’s capsule, may be inconsistently excised, resulting in variable inherent tissue strength. Considering these sources of
variance and to help minimize intergroup variation, a single surgeon was maintained throughout the current study.

For the conjunctival pedicle flap procedure to be considered successful under clinical conditions, the stability of the corneoconjunctival interface provided by the fixation technique must last long enough for permanent adhesions to form through the normal healing process. Although a precise timeline to achieve permanent pedicle flap adhesion is unreported, clinically, the donor tissue appears to be fairly well integrated into the recipient bed by around two weeks post-surgery. Unlike many other locations in the body, as long as the tissues remain coupled, high bond strength may be clinically unnecessary as, when performed properly, conjunctival flaps should rest in the recipient bed with no appreciable tension. Clinically, the concern for excessive tension changes in the post-operative period due tissue contraction, a relatively common phenomenon.\textsuperscript{145,146} Additionally, although objective data is not available, the ocular surface is not likely subject to large amounts of tension under normal conditions. Expected in vivo sources of tension include blinking and normal eye movements, neither of which is likely high in magnitude. These types of forces may be of particular consequence for cyanoacrylate products who have shown good adhesion strength, but poor “peel” strength.\textsuperscript{63} Opening and closing eyelid motion may represent a repeated “peeling” type force leading to flap dehiscence prior to healing. Further research is needed. Additionally, self-trauma due to rubbing the eye undoubtedly exerts substantially more force to the ocular surface than normal physiologic conditions, although utilization of protective measures such as Elizabethan collars can reduce such impacts on surgical repair sites.
Although it was not the primary focus of this study, the time to completion of flap fixation was recorded. The goal of documenting fixation times was to see if one of the liquid adhesive options might potentially achieve a reasonable fixation force, yet exhibit a substantially more rapid fixation time, when compared to suturing. If so, these combined factors might support it as viable alternative to suturing. This might be particularly useful in the clinical setting for patients possessing comorbidities that preclude prolonged anesthesia times. Although the cyanoacrylate and ReSure® were significantly faster fixation procedures than suture, their tensile strength likely remains inadequate and cannot yet be recommended as sutureless alternatives. However, as noted above, there exists no data regarding the objective degree of tensile strength required to maintain conjunctival flap fixation during the healing process.

There are some important limitations to this study that must be acknowledged. In addition to a small sample size, this study utilized ex vivo, post mortem eyes to test conjunctival pedicle flap fixation integrity. Although there is some evidence that adhesives can bond cadaver tissues, it is conceivable that post mortem tissues do not reflect the same integrity or adhesive compatibility as live animal samples. 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 may be the strongest fixation method; glues may be adequate for normal eye movement/loading. Cyanoacrylate specifically may be capable of maintaining adhesion since it withstood substantially more force than the other liquid adhesives. Further studies would ideally test the amount of force placed on a sutured conjunctival flap in vivo. This type of testing poses challenges with the small and delicate tissues of the eye, but may be accomplished
using a method similar to Lee et al. where stretchable and sutureable sensors were used to perform strain measurements on a tendon and ligament in an ex vivo and in vivo porcine leg. Another possibility is adopting a testing method that instead of lap shear (uniplanar tensile testing) may utilize torsional geometry like that of a shear rheometer. This testing setup has shown particular success in measuring tissue bonds of soft adherends (i.e. biologic glues) that are easily damaged and prone to distortion artifact.149

Perhaps the most significant weakness of the current study, was the non-standardized surface area and application method of the different adhesives. Because the fibrin glue is a biologic media, it assumes a permanent role as a scaffold and ultimately becomes incorporated into the host tissue. For this reason, the Tisseel® was placed directly in the keratectomy bed and over top of the perimeter. Both the cyanoacrylate and ReSure® hydrogel sealant are intended to slough off as part of the healing process and as such were only placed superficially over the conjunctival/corneal perimeter. Although the surface area of the conjunctival flaps were reasonably consistent in size (to accommodate fitting into a 6mm recipient bed), their exact measurements were not recorded. This limited us to using peak force, which can vary based on the size/area of the flap. While maintaining a consistent surface area between tests would have been ideal, this study was accomplished to reflect a more clinically scenario. Taking into consideration that increased surface area can withstand higher loading, is arguable that the Tisseel® group was advantaged in this regard, compared to the Resure® and cyanoacrylate groups. However, this did not seem to impact outcomes substantially, as Tisseel® was unable to withstand increased tensile force when compared to all other groups. It is possible that
prior to polymerization while in the liquid state, the glue seeped down into the conjunctival keratectomy border and effectively filled that potential space.

F. Conclusion

Conjunctival pedicle flap fixation using 8-0 VICRYL® suture withstood a significantly greater maximum applied tensile force in comparison with the ReSure®, Tisseel®, or cyanoacrylate adhesives. Tisseel® fibrin glue fixation time was significantly longer than all other methods and ReSure® hydrogel sealant exhibited a significantly shorter fixation time than all other methods.

Although adhesions were achieved in all testing groups, suture material was significantly stronger than the rest. As suture material is considered the current gold standard, it is not recommended to use any of the other adhesives as the sole fixative for conjunctival pedicle flaps. Further research is required prior to moving to a live animal model.
REFERENCES


70. Rodriguez EN, Townsend WM, Stiles J. Double drape tectonic patch with cyanoacrylate glue for surgical repair of corneal defects: 8 cases. *Veterinary Ophthalmology*. n/a(n/a).


Figure 1. Clotting cascade with fibrin adhesive components\textsuperscript{87}
Figure 2. Keratectomy site. In this image, a circular delineation represents the 6mm keratectomy site performed by a restricted depth punch biopsy. Air bubbles are noted in the anterior chamber, as a result of re-establishing normal intraocular pressure via paracentesis of saline into the anterior chamber.

Figure 3. Conjunctival pedicle flap. In this image, a conjunctival pedicle flap is resting in the 6 mm recipient keratectomy bed prior to being secured with an adhesive.
Figure 4. Duploject® fibrin glue application system. This double-barrel syringe precisely combines the components of the clotting cascade.

Figure 5. Representative image of cornea being harvested with a scleral ring
Figure 6. Harvested conjunctival pedicle flap secured with cyanoacrylate

Figure 7. Harvested conjunctival pedicle flap secured with ReSure®
Figure 8. Harvested conjunctival pedicle flap secured with suture

Figure 9. Harvested conjunctival pedicle flap secured with Tisseel®
Figure 10. Sample Attachments to Accelerometer and Potentiometer. In this image, a harvested conjunctival pedicle flap is secured to the upper and lower stages of the tensile testing unit. The cornea is clamped to the upper stage and the pedicle portion of the conjunctiva is secured to the bottom stage prior to testing.
APPENDIX 2: TABLES

Table 1. Summary of peak tensile force statistics

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Table 2. Summary of fixation time statistics

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