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Development of an In Situ Printing System With Human Platelet Lysate-Based Bio-Adhesive to Treat Corneal Perforations

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Purpose: Corneal perforation is a clinical emergency that can result in blindness. Currently corneal perforations are treated either by cyanoacrylate glue which is toxic to corneal cells, or by using commercial fibrin glue for small perforations. Both methods use manual delivery which lead to uncontrolled application of the glues to the corneal surface. Therefore, there is a need to develop a safe and effective alternative to artificial adhesives.

Methods: Previously, our group developed a transparent human platelet lysate (hPL)-based biomaterial that accelerated corneal epithelial cells healing in vitro. This biomaterial was further characterized in this study using rheometry and adhesive test, and a two-component delivery system was developed for its application. An animal trial (5 New Zealand white rabbits) to compare impact of the biomaterial and cyanoacrylate glue (control group) on a 2 mm perforation was conducted to evaluate safety and efficacy.

Results: The hPL-based biomaterial showed higher adhesiveness compared to commercial fibrin glue. Treatment rabbits had lower pain scores and faster recovery, despite generating similar scar-forming structure compared to controls. No secondary corneal ulcer was generated in rabbits treated with the bio-adhesive.

Conclusions: This study reports an in situ printing system capable of delivering a hPL-based, transparent bio-adhesive and successfully treating small corneal perforations. The bio-adhesive-treated rabbits recovered faster and required no additional analgesia.

Translational Relevance: The developed in situ hPL bio-adhesives treatment represents a new format of treating corneal perforation that is easy to use, allows for accurate application, and can be a potentially effective and pain relief treatment.

Introduction

Corneal perforation refers to a full penetrating wound in cornea where all layers of cornea have been damaged. It is a corneal emergency that can lead to corneal melting and blindness. The current management methods include using tissue adhesives: cyanoacrylate and fibrin glue, however, cyanoacrylate glue is toxic to corneal cells.[1] Both fibrin and cyanoacrylate glue showed similar effectiveness in up to 3 mm with corneal perforation treatments at 79% and 86% with a re-application rate of 30%.[2] Cyanoacrylate glue is artificial and does not bio integrate requiring eventual removal. Thus, there is scope for improved adhesives for corneal perforation treatment.

Tissue engineering has previously been used within ophthalmology to generate implants or structures to replace partial or entire eye structures.[3] In recent years, in situ bioprinting biomaterials directly onto injured sites represents a relatively new approach for tissue repair.[4] Using a hand-held 3D printer referred to as a biopen, Duchi et al. were able to deliver cells incorporated within GelMa bioink directly to chondral defects to show early cartilage regeneration.[4] It is possible that in situ bioprinting, the direct deposition of therapeutic biomaterials to corneal injuries, can be an effective treatment option for corneal perforation. The success of this approach will be reliant on the co-development of effective adhesive biomaterials and a compatible printing device.

Our group previously shown by combining human platelet lysate (hPL) with low concentrations of fibrinogen and thrombin, a transparent fibrin-based gel can be formed.[5] Using hPL to treat various ocular surface diseases, such as dry eye syndrome, has previously shown positive results with successful healing and reduced inflammation.[6] The current commercially available fibrin glues contain high concentrations of fibrinogen (40 mg/mL to up to 120 mg/mL) and mostly remains not transparent, making the component highly viscous and incompatible with functional visual acuity during healing.[7,8] Our hPL based bio-glue contains much less of fibrinogen at 2 mg/mL, which mirrors the physiological concentration in blood.[5] Our earlier work confirmed its transparency, adhesion to corneal tissue, and ability to accelerate corneal epithelial cell wound healing. Further, its shear thinning properties make it compatible with an extrusion-based printing processes.[5]

In this current study, we aim to develop an in situ printing system for the application of an hPL based bio-adhesive to treat corneal surface and full thickness wounds. In particular, we examine the rheological behavior of the bio-adhesive including its crosslinking profile, adhesiveness and burst pressure. The generated mechanical data was used to refine the design of an extrusion-based delivery device. As a further step to confirm the potential practical application of the system, a pilot rabbit trial was conducted to evaluate the novel bio-adhesive's safety and effectiveness in sealing small corneal perforations.

Materials and Methods

Generation of hPL Based Fibrin Ink

The hPL-based fibrin bio-adhesive had two parts. Part A consisted of 20% hPL (v/v) and 4 mg/mL human fibrinogen (Merck, USA) in DMEM/F12 (Life Technologies, USA). Part B was made of 10 U/mL human thrombin (Merck) in DMEM/F12. Mixing parts A and B in a 50:50 volume ratio resulted in the formation of a perforation-sealing hydrogel. All ingredients received were sterile and preparation was conducted using aseptic techniques in a biological safety cabinet.

Measurement of Rheological Behavior of the Bio-Adhesive

Rheological behavior was examined by an AR-G2 rheometer (TA instruments, USA) using a 40 mm/2 degree cone plate geometry. Oscillatory tests were performed at 0.1% strain and 0.1 Hz frequency. To measure the effect of shear, viscosity (η) of each part (part A and part B before mixing) was measured across a shear rate range of 0.1 to 500 1/s. Experiments were conducted at room temperature (RT) and 700 µL of each component was used during each experimental repeat. A minimum of three repeats were performed.

Bio-adhesive crosslinking behavior was measured at RT and 34°C (to mimic corneal surface temperature) through an oscillatory time sweep test. Part A (500 µL) was initially added to the sample stage, followed by part B (500 µL). The experiment began immediately after the addition of part B to part A, and storage modulus (an indication of gelation) of the mixed sample was measured over 20 minutes. A minimum of three repeats were performed.

Measurement of Adhesiveness of the Bioink

Porcine skin (Coles, 2246384P) was first scraped to remove the lipid phase and cut into pieces (2.0 cm × 1.0 cm), before soaking in phosphate-buffered saline (PBS) overnight at 4°C. Tissue samples were then glued onto plastic fixtures using cyanoacrylate glue. Part A (40 µL) was first applied to the skin, then 40 µL of part B was added to part A. Finally, another piece of porcine skin was added with an overlapping area of approximately 1.0 cm × 1.0 cm before the sample was incubated at 37°C for 3 minutes. A lap shear test was conducted using the EZ-S mechanical tester (Shimadzu EZ-L) equipped with 10 N load cell and 1 mm minute −1 crosshead speed ($n \geq 3$; Fig. 1). The adhesive strength (E) was calculated using the equation $E = F/S$ (where F is the force at failure and S is the bond area). Artiss glue (Baxter, USA) was used as a control following the same procedure. The test was repeated three times.

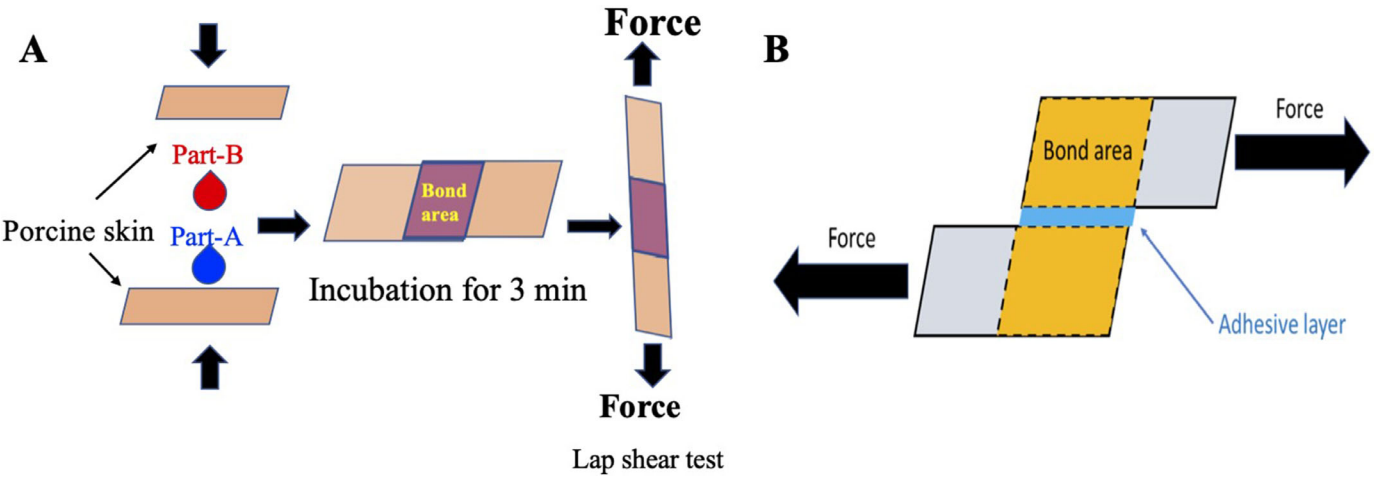

Figure 1. Schematic illustration of adhesion test. (**A**) The pictorial diagram of the set up of the experiment. (**B**) The enlarged details of the adhesion test.

Evaluating Burst Pressure in an Ex Vivo Human Corneal Model

Human corneas not meeting transplantation quality requirements were obtained from New South Wales Organ and Tissue Donation Service (Human Research Ethics Committee approval 14/275). A puncture wound of approximately 1.5 mm in diameter was created centrally in the donor corneas. Corneas were then placed in a Hanna artificial anterior chamber (AAC). The pressure at which perforated corneas with no sealant exhibited stable leaking was measured for each cornea prior to the test, and taken as the baseline pressure. The bio-adhesive ($n = 3$) was administered to the wounds through the printing device for 5 seconds and left for 5 minutes allowing adequate curing prior to burst pressure testing. The ACC was connected to a syringe coupled with a load cell device and actuated by a Shimadzu mechanical tester to record applied force within the fluid system (Fig. 2). Sealant failure was determined when wound leakage through the corneal wound was observed. Burst pressure was calculated as in Equation 1 and recorded in mm Hg.

$$\frac{peak\ force\ experienced\ before\ leakage\ -\ baseline\ force}{surface\ area\ of\ the\ anterior\ chamber} \quad (1)$$

Scanning Electron Microscopy of the Hydrogel Formed by the Bio-Adhesive

Scanning electron microscopy of the hPL and fibrin-based hydrogel was performed by using a JSM-6490LV scanning electron microscope (SEM; JEOL Ltd., Japan). Scaffolds were frozen in liquid nitrogen for 40 seconds and then assessed directly. Images were taken at magnification (× 200 and × 500).

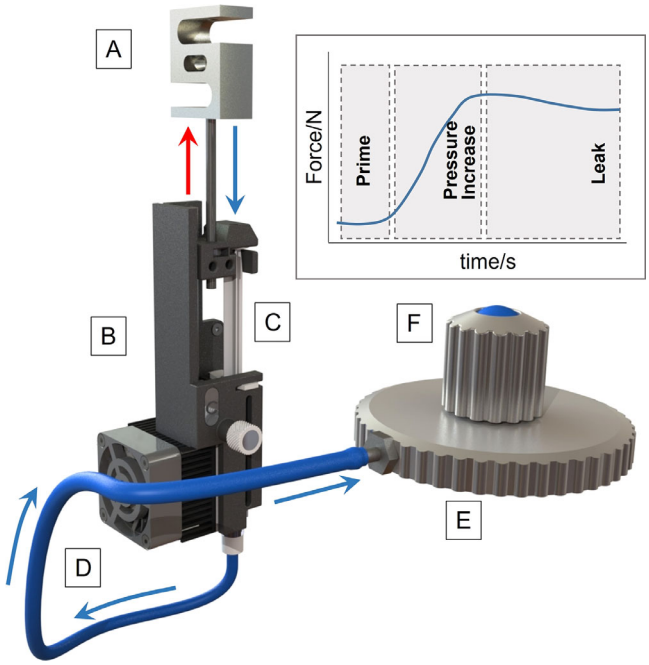

Figure 2. Burst pressure measurement system with inset indicating expected force profile observed during testing. (**A**) Load cell coupled to the Shimadzu mechanical tester. (**B**) The 3 cc syringe mounting carriage rigidly mounted to test base of the Shimadzu mechanical tester. (**C**) The 3 cc syringe containing water. (**D**) Nylon tubing connecting the syringe outlet to the inlet of Hanna AAC. (**E**) Hanna AAC. (**F**) Cornea mounted in Hanna AAC.

Development of Hand-Held Biopen

An electromechanical two-component delivery device (Fig. 3) was developed for controlled mixing

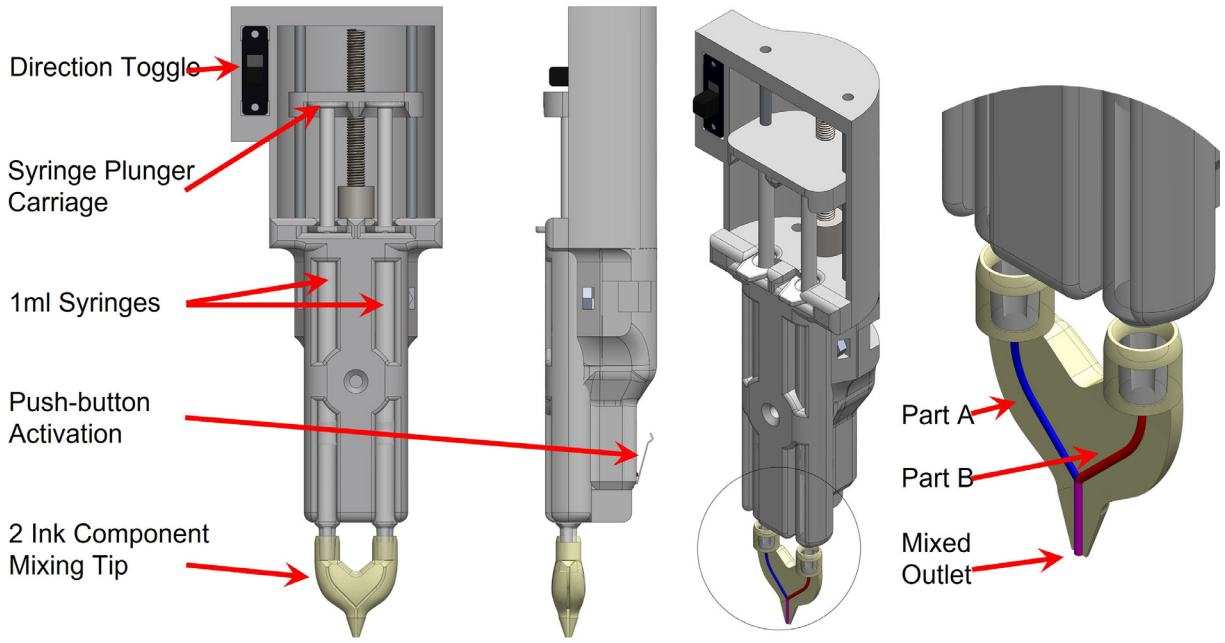

Figure 3. Schematic representation of electromechanical ink delivery device, iFix Pen.

and delivery of part A and part B ink components in a 50:50 ratio at a fixed target extrusion rate of 1 µL/s. Extrusion force was applied to the plungers of the two separate 1 mL syringes via a geared micro-stepper motor (100:1 gear ratio) connected to a 4 mm lead screw with a thread pitch of 0.7 mm. Linear guides on either side of the plunging plate ensured consistent vertical motion. The motor was configured for both forward and reverse directions using a direction toggle switch, allowing for extrusion and subsequent resetting of the plunger position before next use. User control was achieved through depression of the micro switch located at the end of the pen. Continuous depression was required to drive the motor. The rate of motor revolution and in-turn translation of the plunger carriage was controlled by an Arduino Nano and A4988 stepper-motor controller after pressing of the pen activation push button. A test of this printing system on a donor human eye following the ethic requirement was recorded and can be viewed in the supplementary video (Supplementary Videos S1, S2).

In Vivo Corneal Perforation Rabbit Trial

Five female New Zealand white rabbits between 2 and 3 kg were used in the trial. The trial was conducted at the University of Sydney Charles Perkins Centre Laboratory Animal House Facility with Animal Ethics Committee approval (AEC: 2018/1317). The procedure adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Upon arrival, all rabbits were given a complete general health and ocular examination and were excluded if demonstrating signs of pre-existing corneal abnormalities. The rabbits were group housed and underwent acclimatization for a minimum of 3 weeks prior to the study commencement. Rabbits underwent the surgical corneal procedure while under general anesthesia performed by the veterinary staff, followed by standard anesthesia procedures.

The rabbits were divided into two groups to receive management of a surgically induced corneal perforation in one eye only with either bio-adhesive (treatment group), or cyanoacrylate glue (control group). The first group consisted of three rabbits, two treated with bio-adhesive and one received the control glue treatment. The second group received a reduced concentration of povidone iodine (PI; described below) and consisted of two rabbits, one receiving the bio-adhesive treatment and one cyanoacrylate glue. In total, three animals received the bio-adhesive treatment and two received the control glue treatment.

To create a full thickness perforation in the first group, the ocular surface and periocular skin surface region were prepared with 10% PI solution, flushed with sterile saline (0.9%), and dried by sterile surgical spears. A sterile blade (Bard Parker size 11) was used to create a complete perforation of approximately 2 mm in length in the peripheral region of the cornea. The wound was full thickness and was Seidels positive at completion. The bio-adhesive was then extruded over the perforation in the treatment group ($n = 3$) and left

to set for 2 minutes. Commercial cyanoacrylate glue was administered over the perforation in the control group ($n = 2$). A modification of the procedure was made to the second group by dropping 10% PI to 0.2% to minimize cytotoxicity. All wounds were created and treated as described previously.

Following recovery from anesthesia, the rabbits were individually housed and received meloxicam 1.5 mg kg^{-1} subcutaneously daily. Topical chloromycetin eye drops and preservative-free eye lubrication were applied every 4 hours during working hours. Pain was scored every 2 hours by the veterinary staff (independent from this research work) throughout working hours based on a pain score system (Supplementary S1). This was an in-house developed rabbit simple descriptive pain score system derived from a commonly used system for clinical veterinary patients (Supplementary 2). The scale was zero to four, with zero representing the best possible score. If a rabbit was scored three or higher, rescue analgesia was administered (buprenorphine 50 µg kg^{-1} IM or methadone 0.5 mg kg^{-1}). Medication was continued until corneal wound healing was complete.

Corneal imaging (via portable slit lamp) following administration of topical fluorescein in the operated eye occurred four times per day at regular intervals until wounds healed. Complete wound healing was defined as the absence of fluorescein corneal staining following administration. The wound and surrounding area were examined for evidence of postoperative complications. Antibiotic eyedrops were administered following slit lamp examination.

Histologic and Immunostaining Examination

After wound healing, rabbits were monitored daily for side effects. At the 3-week postoperative timepoint, rabbits were humanely euthanized with pentobarbitone IV in accordance with University of Sydney Laboratory Animal Services Protocol. Each rabbit was enucleated bilaterally and the eyes fixed with 4% paraformaldehyde (PFA). Once fixed, corneas were dissected from the globe and then bisected and embedded in paraffin wax. These blocks were sectioned at a thickness of 5 µm and stained with hematoxylin and eosin, as previously published.[9] Histologic examination was performed with light microscopy. Immunostaining using α-smooth muscle actin antibody (SMA, clone 1A4; Sigma, USA) was also conducted on the sections, as previously reported.[9] Briefly, the sections went through antigen retrieval using 0.01 M citrate buffer (pH 6) and then blocked with 5% bovine serum albumin (BSA) in Tris-buffered saline with 0.1% Tween-20 (TBST). The sections were incubated with primary SMA antibody (1 in 1000 dilution) overnight at 4°C followed by incubation with second antibody donkey anti-mouse IgG Alexa 488 (1 µg/mL; Thermo Fisher, USA) and Hoechst nuclear staining (1 µg/mL; Thermo Fisher) for 2 hours at RT and washed in TBST. Slides were mounted in 20% glycerol/PBS, coverslipped, and viewed using the Zeiss LSM 700 scanning laser confocal microscope and image software (Zen 2011; Carl Zeiss MicroImaging GHBH, Germany).

Statistical Analysis

All data generated in this study were analyzed using unpaired Student's t-test using SPSS Statistics (IBM, USA). The P values < 0.05 were considered significant ($^*P \leq 0.05$, $^{**}P \leq 0.01$, and $^{***}P \leq 0.001$).

Results

Rheology Results of Viscosity and Cross-Linking Behavior of a Bio-Adhesive

Both parts of the bio-adhesive showed shear thinning behavior characteristic of a non-Newtonian fluid (Fig. 4A). Viscosity became less dependent to shear rate due to gradual breakage of molecular networks under increased shear force.

Crosslinking behavior of the ink versus time and temperatures was recorded 30 seconds after adding part B to part A. Crosslinking was initiated as evidenced by an increase in storage modulus (G') value, which gradually levels off by time indicating formation of crosslinked networks (Fig. 4B). When measured at 34°C, G' increased dramatically after addition of part B, and crosslinking was almost completed after 2 minutes and 30 seconds. At room temperature, the G' was still increasing after 2 minutes and 30 seconds until the end of the experiment, albeit at a slow rate. The final G' was higher at RT (8.7 Pa) than at 34°C (5.8 Pa).

Physical Property Results – Adhesive and Burst Pressure

The bio-adhesive had an adhesive strength at 1575 ± 195 Pa higher than Artiss (control) at 1260 ± 228 Pa, despite not being significantly different. Mean burst pressure of the bio-adhesive to seal a 1.5 mm perforation was 91 ± 27 mm Hg in the human corneas. This pressure exceeded the mean intraocular pressure of a normal adult eye (approximately 14 mm Hg).[10–12]

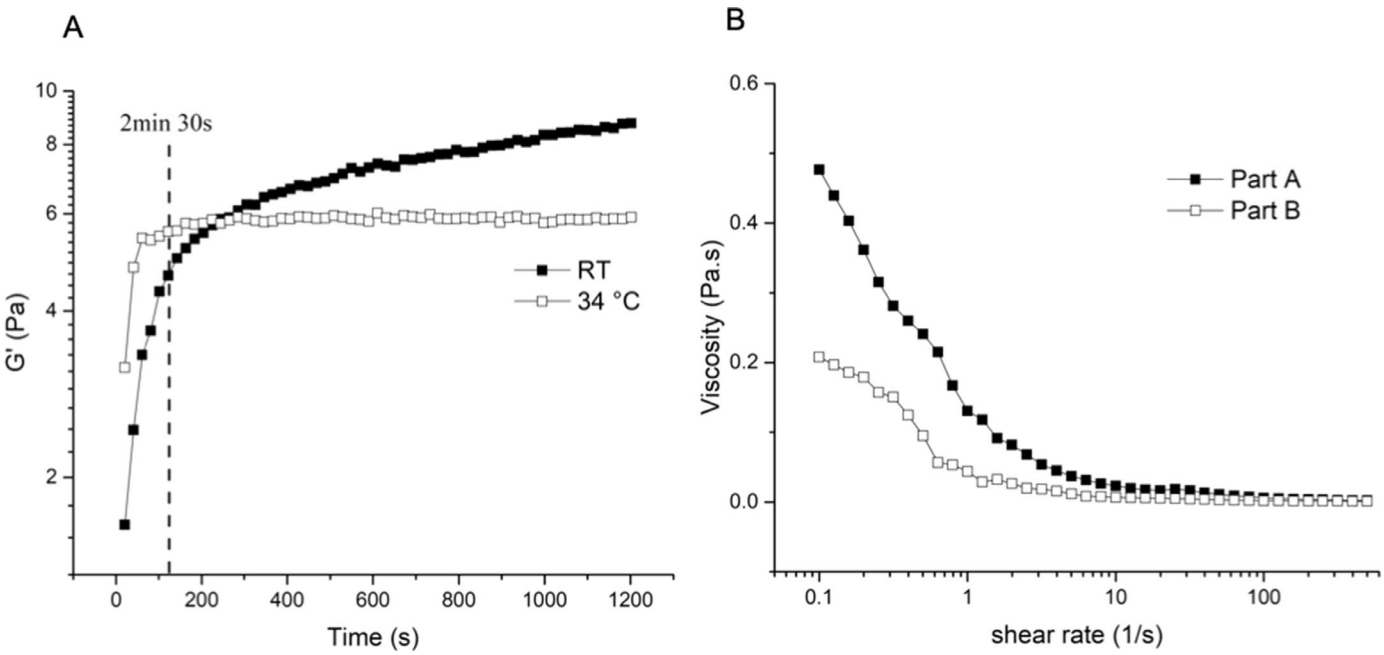

Figure 4. Rheology data showing shear thinning (**A**) and crosslinking profile (**B**). **A** Variation of viscosity versus shear rate for part A (*solid square*) and B (*hollow square*). **B** G′ versus time at RT (*solid square*) and 34°C (*hollow square*).

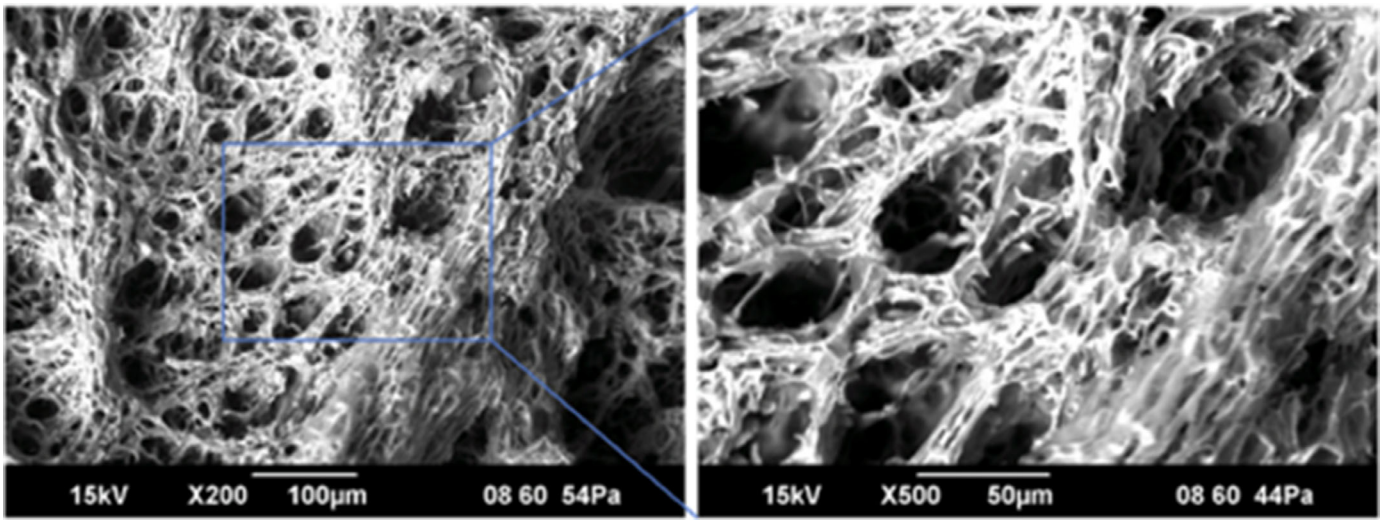

Figure 5. SEM photographs of the hydrogel structure formed by the bio-adhesive.

Microporous Structure of the Hydrogel

Throughout the cross-sectional area, hydrogel formed by the hPL based fibrin bio-adhesive exhibited highly porous, micro-structured interconnected networks that were formed by fibers (Fig. 5). SEM images showed multiple fibrin fibers assembled and bundled together. The diameter of each fiber ranged from approximately 0.1 to 0.5 µm, consistent with diameters of previously reported fibrin fibers.[13]

Extrusion Based Electromechanical Pen for Bio-Adhesive Delivery

Flow rate testing was conducted to determine total output of the delivery device. One syringe filled with water was driven for 30 seconds, and the deposited volume of water measured. Measured flow rate over six trial replicates was 1.02 ± 0.03 µL/s and effective within a 5% tolerance of the target volumetric output. This trial was then performed using the two component inks

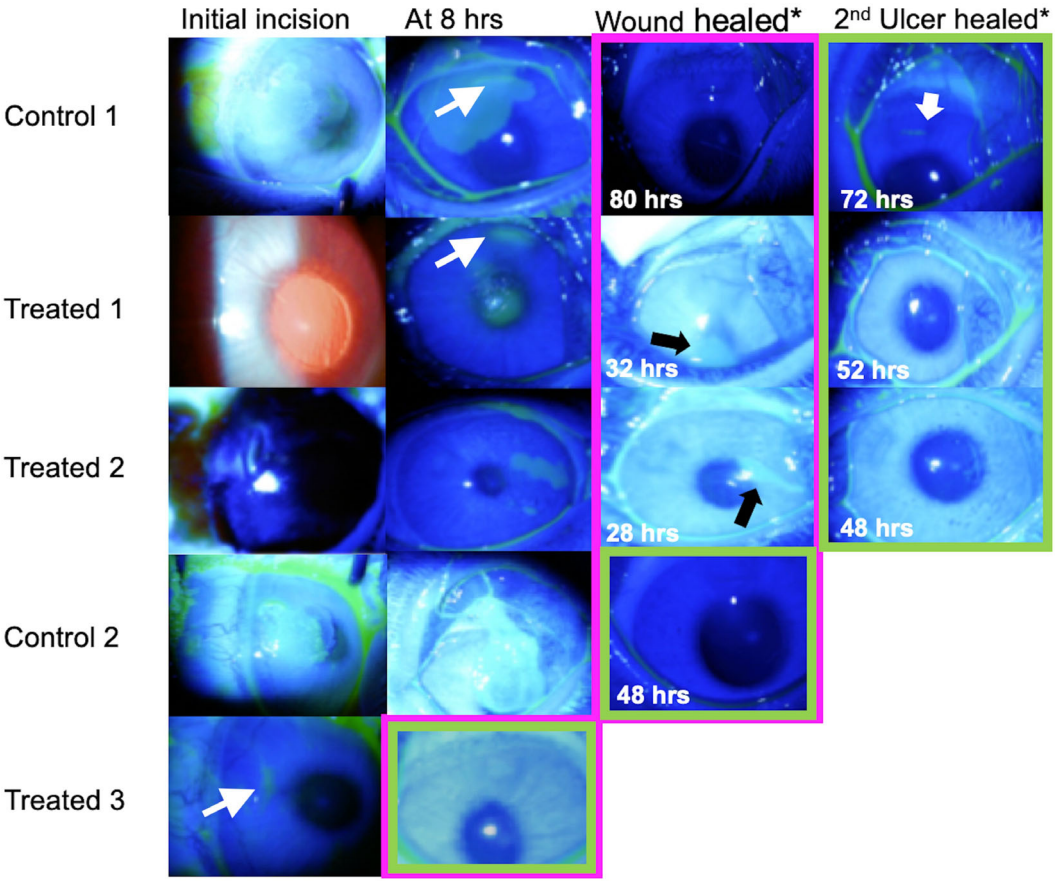

Figure 6. Corneal perforation tests with two controls and three bio-adhesive treated eyes. All bio-adhesive treated eyes healed much quicker than control corneas. Control 2 and treated 3 were conducted with modified surgical procedures and whereas the treated three cornea had no complications and healed six times faster than the controls. Pink rectangle boxes indicate the time that perforation healed. Green rectangle boxes indicate the time that the ulceration was considered healed. White arrows: Traces of corneal incisions. Black arrows: Ulcerations. Note: Some corneas had secondary ulcerations which healed before the perforations.

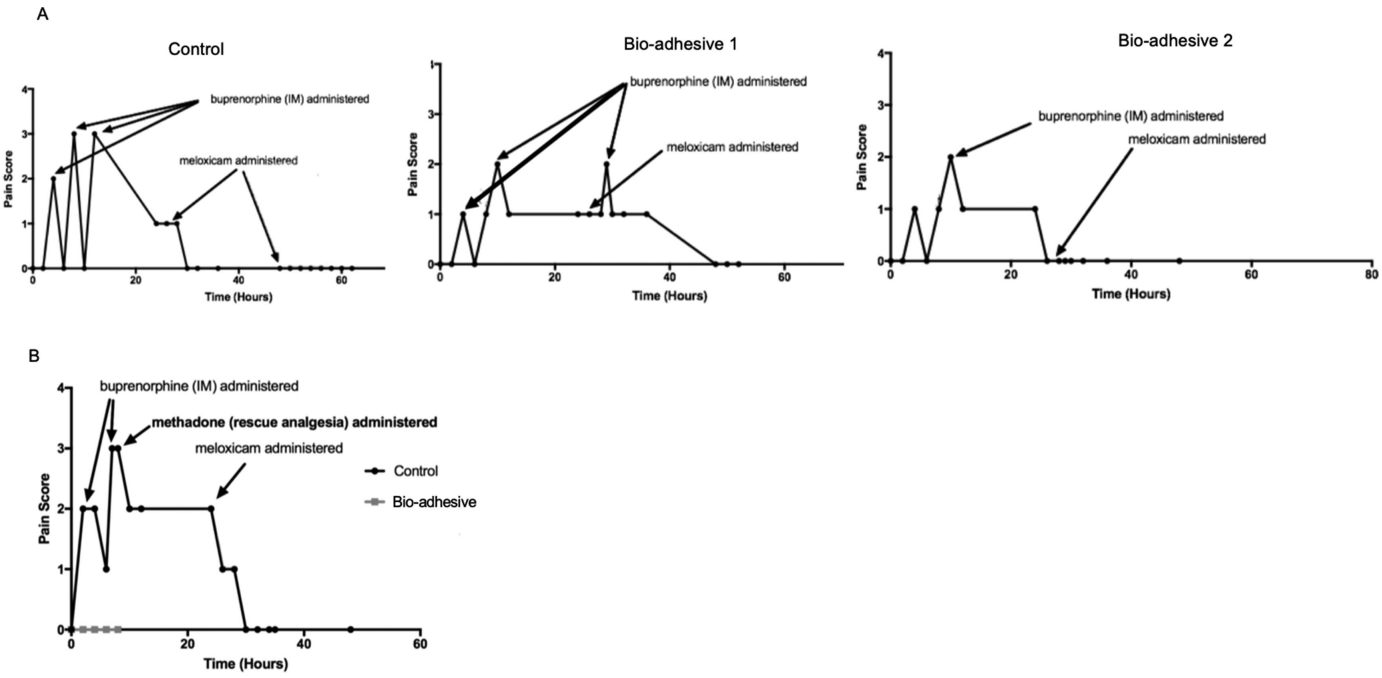

Figure 7. Pain score and analgesia administration. (**A**) First round of rabbits. (**B**) Second round of rabbits.

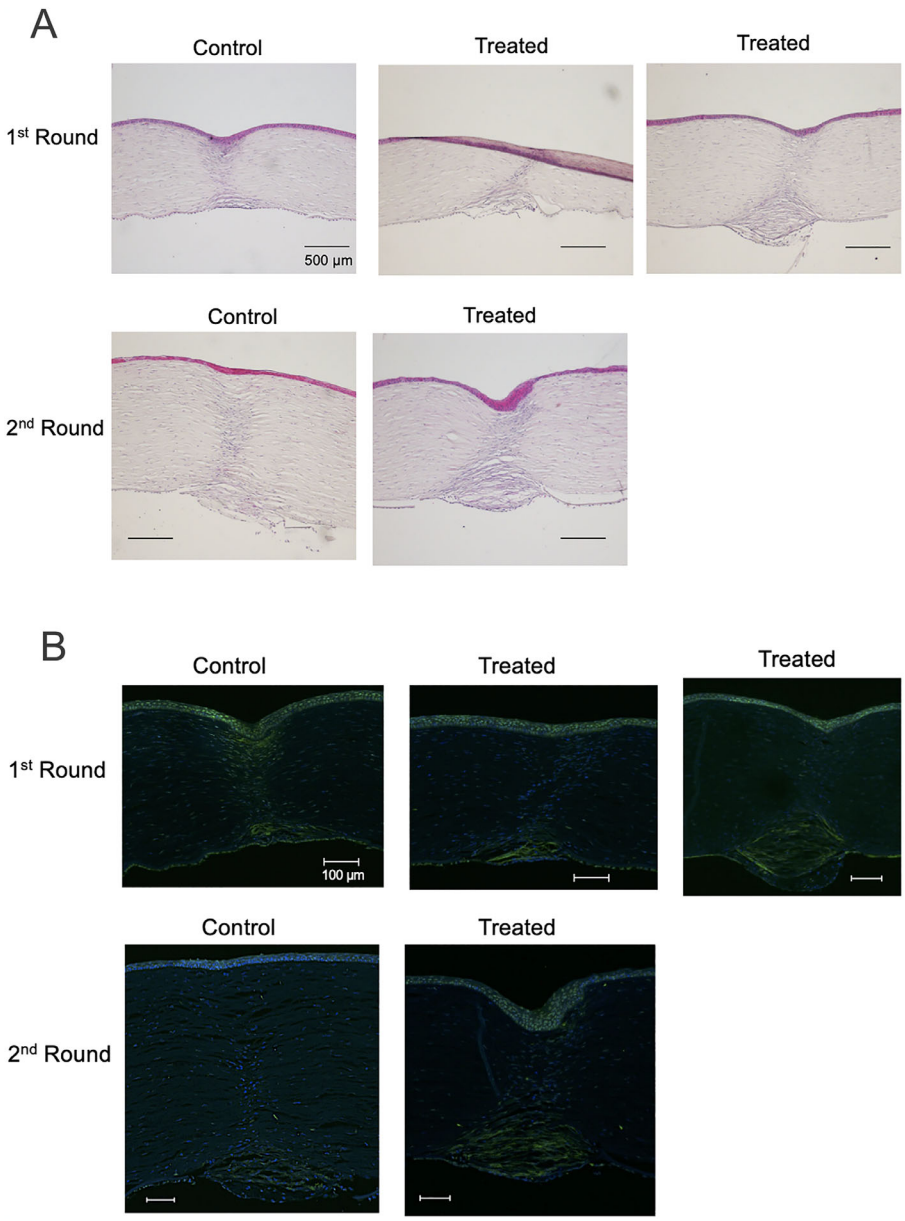

Figure 8. H&E and SMA staining of perforation regions. (**A**) H&E staining and (**B**) SMA immunostaining with green being SMA and blue being nuclei staining.

with a target flow rate of 0.5 µL/s for each ink. Resultant mean extrusion rate was 0.512 µL/s with a deviation $< \pm$ 0.01 µL/s showing very small discrepancy.

In Vivo Test Results on Healing and Pain Assessment

The bio-adhesive treated rabbits (treated 1 and 2) in the first group healed approximately three times faster than control group (control 1, Fig. 6, pink box). However, all rabbits in this group developed secondary corneal ulcers, with bio-adhesive treated rabbits showed milder symptoms, including moderate conjunctival inflammation compared to cyanoacrylate glue which developed into severe conjunctivitis and chemosis. In addition, the secondary corneal ulcers healed more quickly in the bio-adhesive treated rabbits compared to controls (see Fig. 6, green box). The second group test was conducted with a reduction of PI (0.2% versus original 10%) was made to reduce potential cytotoxic when preparing the ocular surface for surgery after discussion with the veterinarians. The original operation protocol was adopted from human practice; however, rabbit corneas could have different tolerance to PI compared to humans.

With the modification, the bio-adhesive treated cornea (treated 3) showed no macroscopic evidence of inflammation or complications, whereas the control cornea (control 2) still developed a second corneal ulceration (see Fig. 6). The corneal perforation treated with bio-adhesive healed at 8 hours postoperatively, 6 times faster than the control cornea (see Fig. 6B). Tracks of corneal incisions can be seen in the rabbit corneas as fine fluorescence green lines before healing (see Fig. 6). Corneal leakage was not detected in any rabbit. All time point images can be found in Supplementary 2.

In the first group, bio-adhesive treated rabbits had lower pain score and received less rescue analgesia in comparison to the control rabbit (Fig. 7A). In the second group, no additional analgesia was required for the bio-adhesive treated rabbit, whereas the control rabbits required an additional four doses of rescue analgesia postoperatively (Fig. 7B).

The hematoxylin and eosin microscopic analysis showed similar tissue structures across all corneas harvested 3 weeks after surgeries. Both bio-adhesive and control corneas showed a regenerated epithelial layer and stromal scar formation shown as a dense stromal structure compared to the surrounding normal tissue (Fig. 8A). The SMA staining showed similar results between control and bio-adhesive corneas (Fig. 8B). The control had higher SMA staining in the first round compared to the treated group, however, in the second round, the control had weaker staining compared to the treated sample. Most SMA stainings were found in the endothelial regions. No statistical analysis was conducted due to the small numbers.

Discussion

In this study, we successfully developed an in situ printing system using an hPL based bio-adhesive to treat small corneal perforations. The bio-adhesive used was an hPL-based fibrin biomaterial previously developed by our group which showed high transparency and cell compatibility.[5] Our bio-adhesive was effective, indicating a six times faster healing rate and zero pain score when compared to the control group (cyanoacrylate glue).

The crosslinking profile of the bio-adhesive showed that gelation started immediately after mixing, and full cross-linking achieved around 2 minutes at 34°C (the surface temperature of cornea). The microstructure of the hydrogel formed showed an organized porous structure formed by fiber bundles, which was different to published fibrin gel structures that have found single fibrin fibers interconnected in a mesh format.[11] We also compared its adhesiveness to the Artiss glue and showed that our bio-adhesive had a higher average of adhesiveness strength, albeit the difference did not reach statistical significance. In corneal treatments, Artiss glue is often used to seal small perforations, thus this outcome indicated the potential that our bio-adhesive may be used in a similar manner. It is worth noting that the adhesiveness was conducted on porcine skin for comparison purposes. Human corneal tissue would likely represent a more appropriate surface to measure the true adhesiveness of the biomaterials developed for the cornea.

A hand-held extrusion device was designed based on the mechanical properties of the bio-adhesive to have an extrusion rate of 1 µL/s. This extrusion rate was indicated to provide sufficient operation time for surgeons to apply the ink to the wounded site whereas minimizing the risk of the bio-adhesive clotting in the nozzle. In this trial, the device successfully delivered the bio-adhesive to the injured sites each time. The bio-adhesive solidified in 2 minutes, and remained on the cornea even after instillation of antibiotic eye drops with no leaking identified.

As a first trial of this system, the aim was to demonstrate the usability of the bio-adhesive and delivery device by surgeons and to evaluate its safety and efficacy compared to standard clinical care in a rabbit model. The size of the perforation was at a 2 mm slit cut, which was small and therefore was a relative limitation of this study. A small circular trephine of the cornea would be the next procedure to trial the effectiveness of the bio-adhesive and be a better comparator to cyanoacrylate and fibrin glue. It is worth noting that the burst pressure test was performed successfully on a 1.5 mm circular wound which showed higher adhesive strength than fibrin glue, and withstood burst pressure approximately 6.4 times higher than routine intraocular pressure.

The rabbit trial has a small sample size of five rabbits, and was also divided into two sets due to the surgical procedure modification of reducing PI concentration due to unforeseen corneal toxicity. The initial PI amount (10%) used was adopted from human procedures, however, corneal toxicity and corneal ulceration was observed in all rabbits. A recent publication which showed that instilling 5% PI even for 1 minutes was toxic to rabbit cornea and corneal epithelial defects were induced.[14] The PI was reduced to 0.2% and the second ulceration was not detected in bio-adhesive treated rabbits but was still observed in the control group. This confirms the expected toxicity of cyanoacrylate and the better safety profile of the bio-adhesive.

In addition, despite the small perforation, in all rabbits, the bio-adhesive treated corneal surface healed more rapidly compared to controls treated with cyanoacrylate glue; and all bio-adhesive treated rabbits had lower pain scores than the control rabbits. In the last two rabbits with the modified surgical technique, the bio-adhesive treated cornea showed imminent advantages to cyanoacrylate glue with the wound completely healed 8 hours postoperatively, 6 times faster than control. This rabbit had a consistent pain score of 0 with no additional rescue analgesia required. The results were consistent in showing that the bio-adhesive could lead to faster healing, alleviate pain caused by corneal injuries, either directly or indirectly, by avoiding the conjunctival inflammation induced by cyanoacrylate glue. Scar structures can be seen in all injured areas where the corneal dome shapes were lost and the endothelium did not regenerate into a single layer. Intense SMA staining were detected in the region, indicating the keratocytes had been activated into fibroblastic cells to facilitate stromal injury recovery. These results showed that none of the treatments led to full regeneration of the region, yet they served as an aid for scar formation. All corneas were harvested 3 weeks after surgery and no complications were observed in the chronic phase of the study once the corneal perforations had healed suggesting longer-term potential.

A number of alternative treatments have been published as alternatives to treat corneal perforations including using collagen-based fillers,[15] fibrin glue-assisted amniotic membranes,[16] and LiQD corneas.[17] These studies have demonstrated successful sealing results. The LiQD corneas also contained fibrinogen and thrombin, similar to the fibrin glue-assisted amniotic membrane approach to allow for in situ gelation. Both the collagen-based filler and LiQD cornea required additional steps, including the addition of LP-PEG, and DMTMM to aid gel formation. Increased temperature to 50°C for mixing DMTMM was required in the LiQD method. Our approach required no additional chemical crosslinker and represents an in situ printing system, which is a completely different approach. The extrusion-based device allows in situ gelation as per the LiQD treatment, but also allows in situ printing with no implant or membrane required. This relatively simple model increases the practical potential of the device, in particular in developing countries that may not have access to trained medical personnel. In addition, both studies using CLP-PEG and LiQD did not report pain assessment. We have identified obvious pain reduction in rabbits treated with our bio-adhesive. Reducing pain and discomfort following treatment in the human patient would represent a potential significant advantage. Furthermore, as no additional chemical crosslinking was used and no requirement of high temperature, our bio-adhesive has potential to incorporate bioactive molecules, such as growth factors, antibiotics, and even human cells. Further studies exploring these options are planned.

This study demonstrated a successful treatment of a 2 mm corneal perforation in a rabbit eye using an in-house developed in situ printing system with a transparent hPL based bio-adhesive. Compared to the current standard treatment of cyanoacrylate glue, it led to quicker healing times, less secondary complications, and less analgesia requirements, which is a factor not commonly addressed in animal trials but critical for animals and humans. The bio-adhesive composition was simple with only three active ingredients which are human-derived and clinically safe reagents. The delivery system is a hand-held device, which is easy to use. In conclusion, this combined device and bio-adhesive represents a potentially simple, effective, and easy to use alternative to cyanoacrylate for the treatment of corneal perforations. Due to the physiological compatible fibrinogen included in the formulation, it is also possible that this developed bio-adhesive can be used as a bioink for cell printing. Further tests are required to access this possibility.

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Conflict of Interest: The authors alone are responsible for the content and writing of the paper.

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Supplementary Material

Supplementary Video S1. A video recording on the delivery of the bio-adhesive to a donor eye.

Supplementary Video S2. The uploaded video alongside with two images showed the printing process of two layered lattice structures using the bio-adhesive.