Journal of
Biomedical Optics

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Strong bonding of corneal incisions using a noncontact fiber-optic laser soldering method

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Abstract. Suturing of corneal incisions requires significant skill. We demonstrate a noncontact method that will simplify the bonding process. 5-mm-long penetrating vertical and slanted incisions were made in corneas of eyes, extracted from dead piglets. A fiber-optic laser system was used for laser soldering of the incisions, under close temperature control, using albumin solder. The burst-pressure P_B immediately after the soldering was found to be $P_B \approx 92$ and 875 mmHg, for vertical and slanted incisions, respectively. $P_B = 875$ mmHg is an exceptionally high figure, ≈10 times the clinically acceptable value for sutured incisions. Laser soldering was then performed on penetrating incisions made in the corneas of live healthy piglets, of weight ≈10 Kg. After a healing period, the eyes were extracted, and the corneas were examined by histopathology and by optical coherence tomography. Our method immediately generated watertight and strong bonding without noticeable corneal shape distortion. These results would be beneficial for cataract surgery and for corneal transplantations. The fiber-optic system makes it much easier to bond corneal incisions. In the future, laser soldering could be automated and efficiently used by less experienced surgeons, thereby reducing the workload on the experienced ones. [DOI: 10.1117/1.JBO.24.12.128002]

Keywords: infrared; laser soldering; laser bonding; albumin; cornea; optical fibers.

Paper 190279RR received Aug. 21, 2019; accepted for publication Nov. 11, 2019; published online Dec. 28, 2019.

1 Introduction

The closure of incisions in tissues involves passing a needle through the tissues, close to the edges, pulling them together and relying on tissue and suture strength to hold them tight. Suturing is a delicate, demanding, and time-consuming procedure that requires significant technical skill.[1,2] Sutured sites have an increased risk for leaks, infections, and scarring.[3,4]

Laser-tissue bonding has been introduced in an attempt to solve these problems. There are two methods of laser-bonding of incisions,[5–7] which differ by their biochemical mechanisms: photothermal bonding and photochemical bonding.

(A) Photothermal bonding. There are two basic photothermal procedures: laser welding and laser soldering. In laser-welding, a spot on the approximated edges of an incision is heated by laser light absorption in the tissue or in a coloring agent (which changes the penetration depth of the light).[8–10] The spot is heated to temperature T for time t, causing adhesion. The full length of the incision is then bonded, spot by spot. In laser-soldering, a biological bonding agent, such as, albumin or chitosan,[11,12] is applied at the approximated edges and heated spot by spot. The heating denatures proteins and links them with collagen, thus generating a clot that seals the incision.[13]

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Laser bonding, and specifically the introduction of a biological agent (e.g., albumin), generates a watertight seal and accelerates healing with very little scarring. In addition, it improves the immediate mechanical properties (e.g., tensile strength) of the target tissues after bonding. During the last few years, there has been significant progress in the development of laser bonding methods. Photothermal bonding has already been demonstrated in animal[14,15] and clinical[9] works.

(B) Photochemical bonding. A chemical dye that is applied at the incision is sensitized by visible laser light. The irradiation drives a chemical reaction that initiates bonding through protein crosslinking.[16–18] With this approach, significant bond strengths have been achieved in many tissues. Photochemical bonding has also been demonstrated in animal experiments (including ones on the cornea) and clinically.[16,19]

These two bonding methods have a potential of being widely used clinically. But suturing is obviously the standard method still used for closing incisions.

Our analysis of previous works showed that many researchers calculated the heat distribution generated by laser heating, under various experimental protocols, using different lasers, heating setups, and solder compounds.[20–22] However, very little work was done on monitoring and controlling the temperature of each heated spot on the incision.

The lack of temperature control caused underheating or overheating, and in both cases, the bonding strength was insufficient. Attempts have been made in the past to develop such a control, but more work is still needed. One pioneering work had a camera adapted for temperature-control of laser bonding and was successfully used clinically.[9,14] Yet, none of these approaches has been widely used.

We developed a fiber-optic system that used a middle-infrared (mid-IR) detector to measure the temperature (T) of the heated spot, during the irradiation time (t). A feedback loop was used to control the laser output so that T was kept constant. We concentrated on laser soldering and used albumin as a solder. In addition to the experiments, we also carried out theoretical calculations of the temperature distribution during irradiation.[20,23–27] We tested our calculations at different values of T and t and found optimal conditions for $T \approx 60°C$ and $t \approx 10$ s. This was also verified experimentally, and strong and watertight bonding, without noticeable thermal damage, was obtained.[28]

Using this information, we succeeded in soldering incisions *in vitro* in skin,[29] bowel,[30] dura,[31] and trachea.[32] In experiments on skins of large farm pigs *in vivo*, the wound healing was faster than that obtained after standard suturing, and there were practically no scars.[33] Clinical laser-soldering protocols were also carried out on incisions in the abdomen skins of patients, after laparoscopic cholecystectomy. Again, strong bonding with very little scarring was obtained.[24,34]

Recently, we used robotic laser soldering for bonding incisions in mouse skins *in vitro* and obtained a high bond strength.[35] Using this technique, we were able to deliver a measured amount of heat for a predefined amount of time, repeatably, thereby obtaining standardization and allowing for remote (e.g., daVinci System) operation or even an unaided one.

Suturing is a standard procedure in cataract treatment, corneal surgery, or corneal transplantation (penetrating keratoplasty). It is inexpensive, reliable, and readily available.[3] However, the tissue is very delicate and presents a considerable challenge. One complication in corneal transplantation is astigmatism, due to nonuniform tensions generated by the sutures around the cut, resulting in corneal deformation.[4] Chemical adhesives have also been used in corneal transplantation, but they can have inadequate tensile strength, they are toxic, and suffer from other problems.[36,37]

In our previous experiments on laser soldering of corneal incisions in piglets, we proved that our noncontact and temperature-controlled soldering method, based on a CO_2-laser, generated an immediate, reproducible, and watertight bond.[21,29,38]

One goal of the current research was to maximize the burst pressure P_B of soldered corneal incisions. The second goal was to check if there is any change in the corneal shape of the piglets due to the soldering process. To fulfill the first goal, we found the optimal conditions needed to generate immediate very high P_B ex vivo. We report here these optimal conditions. To achieve the second goal, we used these optimal conditions, assuming that the same P_B will be obtained in live piglets. We allowed the eyes to heal for 6 weeks and observed the corneal shape, using optical coherence tomography (OCT) and histopathology. We did not observe changes in the shape of the cornea as a result of the soldering. Obtaining immediate strong bonding on corneal incisions, without deforming the retina, will be an important step toward bringing laser soldering of corneal incisions from the laboratory to clinical use.

2 Materials and Methods

2.1 *Choice of Wavelength*

The choice of laser wavelength that generates strong bonding greatly depends on the penetration depth of the laser radiation into the tissue. In the past, we used a CO_2 laser, emitting at 10.6 μm, for heating spots on the incision. However, the penetration depth at this wavelength is only a few microns, and therefore the bonding of the incisions was not very strong. In this work, we used a semiconductor disk laser emitting 0 to 0.5 W at 1.9 μm (Fraunhofer Institute, Freiburg, Germany).[39] The radiation at this wavelength is fully absorbed within few hundred microns, so it heated the full thickness of the cornea. We previously established[24] that this operation generated strong bonding while ensuring no retinal damage.

2.2 *Beam Delivery and Temperature Control*

We developed a fiber-optic laser soldering system with an improved handpiece, combining two types of optical fibers arranged in a bundle. The bundle included a silica fiber, surrounded by 4 to 6 AgClBr fibers that had been made by the Applied Physics Group at Tel-Aviv University[24,35] and were highly transparent in the mid-IR. The silica fiber transmitted the 1.9-μm laser radiation from the proximal end to the distal end of the bundle to heat a spot of diameter of ≈2 mm on the incision. The heated spot emitted mid-IR black-body radiation that was proportional to temperature T. The AgClBr fibers transmitted the black-body radiation, from their distal ends to their proximal ends. A mid-IR detector (Infratec LME-335) was connected to the proximal ends, and it detected the radiation and generated voltage V, which was also proportional to the spot T. V was used in a feedback loop to control the laser intensity, keeping T constant. The speed of update of the laser intensity was ~200 Hz. The optimal conditions for laser bonding had already been determined in previous studies and were $55 < T < 65°C$ for $8 < t \leq 12$.[20,28] In this study, we used only these values.

2.3 *Solder Preparation*

The solder was prepared from 67% w/v bovine albumin solution (Sigma Chemicals, St. Louis, Missouri) in doubly distilled water.

2.4 *Surgical Protocols*

By observing the process through a microscope (Fig. 1), the surgeon followed these protocols:

1. A linear incision of length ≈5 mm was made on the cornea. Each incision was made in one of two modes: (I) perpendicular to the corneal surface [Fig. 2(A1); this cut was used only in the *ex vivo* experiments], or (II) slanted with respect to the corneal surface [Fig. 2(A2); this cut was used both in the *in vivo* and the *ex vivo* experiments].
2. Only for the laser-soldering cases: a layer of solder of thickness ≈500 μm was spread over the approximated edges of the incision and some solder was spread between the edges.

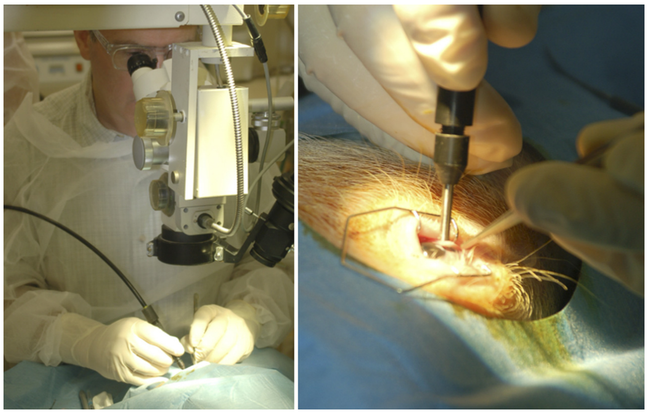

Fig. 1 The laser soldering protocol, using the laser soldering system.

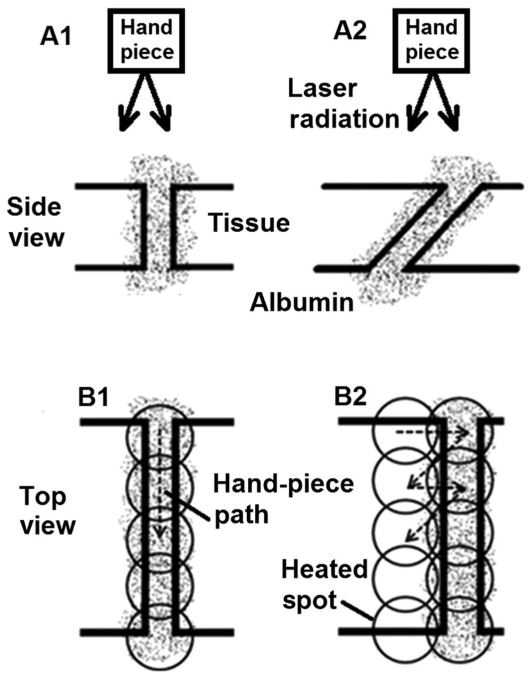

Fig. 2 Soldering protocols: (A1) Cross section of a perpendicular (vertical) incision. (A2) Cross section of a slanted incision. (B1) Top view of a linear heating along an incision. (B2) Top view of a zigzag-pattern heating along an incision. The dotted area marks the places where albumin was applied. The circles mark the heated-spot diameter. The broken arrows mark the direction of the heating spot by spot. This figure is not in scale, the cut edges have been moved far apart, for clarity.

3. The distal tip of the handpiece was brought to a height 3 to 5 mm above the incision, and the laser beam heated a spot on the incision to the optimal values of T and t. The tip was then moved to a neighboring spot along the incision, according to one of two patterns: (I) linear heating [Fig. 2(B1)] or (II) zigzag heating [Fig. 2(B2)]. During our experiments, we used for example a vertical incision-zigzag heating protocol or a slanted incision-linear heating protocol. In most cases, we used a slanted incision-zigzag heating protocol.

2.5 Ex Vivo *Bonding*

The first part of the research consisted of *ex vivo* experiments on eyes, freshly extracted from dead piglets (<24 h). Linear incisions were made in each of these corneas, and the incisions were then laser soldered. We checked the condition for obtaining optimal P_B. These included the optimal laser wavelength, the irradiation temperature T, the time t, and the surgical protocol itself. The optimal conditions were then used for *in vivo* experiments on live piglets.

2.6 In Vivo *Bonding*

The *ex vivo* protocol (Sec. 2.4) that generated the best P_B was selected for the *in vivo* application. The piglets were divided into three groups. In the laser soldering group, the albumin solder was spread both inside and over the incisions, before the heating. In the laser welding, no solder was used. In both cases, the edges of each incision were approximated, and laser bonding was performed under close temperature control. The piglet-eyes of the third, control group, were incised, and left to self-seal and self-heal.

2.7 *Testing the Quality of the Bonding and the Corneal Shape*

In this work, we did not test the thermal damage. However, in previous studies on porcine corneas, we observed that none of the features that characterize damaged tissues (e.g.,, different degrees of coagulation) was visible by histologic or microscopic observations.[6,40]

The following methods were used to assess the quality of the bonded incisions in the corneas.

1. Burst pressure (P_B): Water was introduced into each eye through a hypodermic needle and the water pressure was gradually increased till, at some pressure, a leak was detected somewhere on the organ. This pressure was then logged and called P_B. This P_B was measured by a Lutron PS9302 pressure transducer.
2. OCT: The corneas with bonded incisions were scanned with an OCT apparatus (Visante OCT, Carl Zeiss Meditec, Jena Germany).[27] This method was also used to evaluate the radii of curvature of the corneas, and to compare the results with ones obtained for corneas of eyes that had not been used in our experiments.
3. Histopathology: The same corneal samples were used for histopathological evaluation. The eyes were fixed in formalin 4%, embedded in paraffin, and sectioned through the soldered incision area. Hematoxylin and eosin (H&E) staining was applied before evaluating them by light microscopy.

2.8 *Animal Treatment*

2.8.1 *Before surgery*

Twelve piglets were grown in the Lahav Research Institute (LRI), each weighing ≈10 Kg. They were anesthetized by an intramuscular ketamine and xylazine mixture 0.1 ml/100 g at 3:4 ratio. Afterward, 5% polydine solution was spread on the ocular surface and the surrounding skin, to sterilize the surgical area. The surgical protocols were then performed, under sterile conditions, with the aid of an operating microscope (Inami, Japan). In each piglet, a short, linear incision of length ≈5 mm was made in the cornea of the left eye, near the limbus, and the slanted incision-linear heating protocol was used.

2.8.2 *After surgery*

After the experiments, the piglets were kept at the LRI for 6 weeks, for postsurgery inspection and analysis. Then, they were euthanized and the left eye (the one with the incision) of each piglet was surgically removed and sent to the Tel-Aviv Sourasky Medical Center. There, an OCT system was used to obtain a cross-section image of each of the corneas at the incisions. The eyes were then placed in formaldehyde and sent to the Sheba Medical Center for Histopathology. Our work was performed under the guidelines of the Helsinki Accord, authorization number 01-16-026, the State of Israel, and Tel-Aviv University.

3 Results

3.1 Ex-Vivo *Experiments*

Several soldering protocols were tested (Fig. 2) for soldering of incisions on the cornea. The results (Table 1) showed that for the slanted incision-zigzag heating protocol the average P_B for 14 eyes was (875 ± 274) mmHg. It is an order of magnitude higher than the average value of (92 ± 8.4) mmHg, obtained for 18 eyes, where the vertical incision-linear heating protocol (Table 1) was used. The latter is also higher than the clinically accepted value of $P_B = 80$ mmHg for sutured corneal incisions.[21,37]

We also did laser welding of incisions *ex vivo*: we used either one of our heating protocols, without any solder, as preliminary experiments in preparation for the *in vivo* experiments. The average P_B measurements in these cases (not shown) exhibited bond strengths of $\sim 30 \pm 10$ mmHg in 15 porcine eyes, which is far below the acceptable value of 80 mmHg.

We compared our results by calculating the one-sample T-test of each experiment with the accepted value of 80 mmHg for suturing. We obtained P-values that are smaller than 2%, 0.1%, and 0.01%, for the soldering methods 1, 2, and 3, as shown in Table 1, respectively. Calculating the unpaired two-sample T-tests for either permutation of the average P_B of any two of these bonding methods, yielded P-values < 0.01. These results are statistically significant.

OCT and histopathology of the soldered incisions in these eyes showed well-approximated tissues, and no noticeable corneal shape distortions at the area of the cut (not shown).

Furthermore, qualitative comparisons of the radii-of-curvature of corneas soldered by our protocols, to those that had been welded, were carried out using OCT and histopathology imaging. They exhibited no noticeable changes in the radii of curvature, suggesting no or minimal generation of optical aberrations to the cornea by the laser soldering protocol. These are not shown for the *ex vivo* results but are shown representatively for the *in vivo* results, in Fig. 3.

3.2 In-Vivo *Experiments*

The success in immediately achieving high P_B after soldering in the *ex vivo* experiments paved the way for obtaining permission for carrying out the *in vivo* experiments in live piglets. In these, we used the temperature-controlled laser-bonding of corneal incisions. Maintaining the bonding protocols, we considered it safe to assume that P_B values in the *in vivo* and in the *ex vivo* experiments, immediately after the surgery, will be similar. Our next stage was to laser-solder incisions in the corneas of live piglets, followed by a postoperative follow-up on the bonded incisions.

Table 1 Burst-pressure (P_B) measured after *ex vivo* soldering of incisions of length ≈(5 ± 1) mm in porcine corneas, using different soldering protocols.

ID	Bonding method	Eye ID	P_B (mmHg)	Eye ID	P_B (mmHg)	Eye ID	P_B (mmHg)	P_B (mmHg) average
1	Make a vertical incision, spread albumin, solder the cut in a linear motion.	1	69	4	100	7	83	89.5 ± 10.4
		2	84	5	98	8	96	
		3	91	6	95			
2	Make a vertical incision, spread albumin, and solder the cut in a zigzag motion.	9	83	13	98	17	97	92 ± 8.4
		10	81	14	81	18	85	
		11	103	15	96			
		12	99	16	95			
3	Make a slanted incision, spread albumin, and solder the cut in a zigzag motion.	19	1025	24	1350	29	626	875 ± 274
		20	1024	25	650	30	800	
		21	1020	26	420	31	660	
		22	1130	27	644	32	846	
		23	1300	28	754			

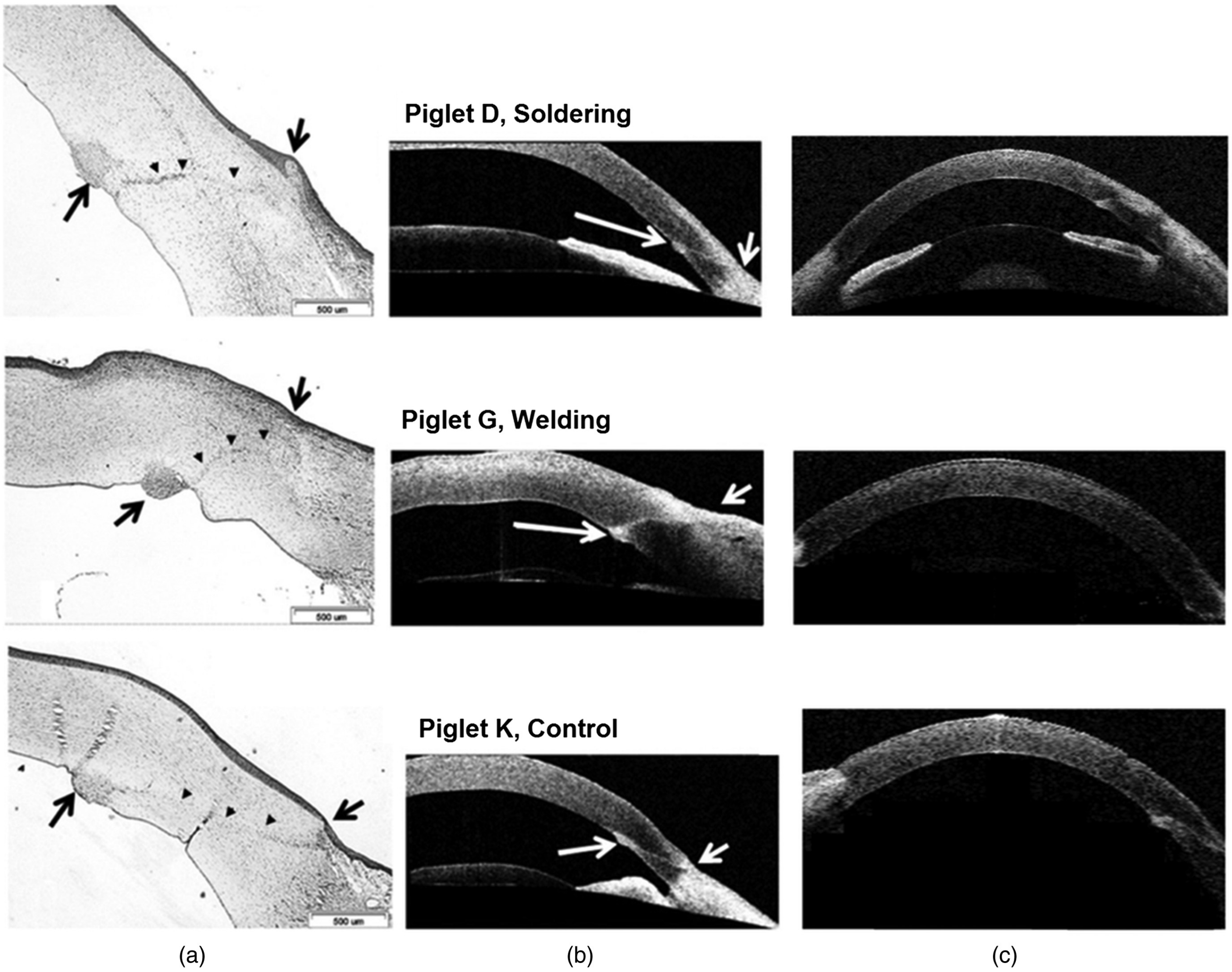

Fig. 3 Each row shows three images of a cross section of a bonded incision: (a) histopathology, (b) OCT at high magnification, and (c) OCT at low magnification. The top row represents a soldered incision in the cornea of piglet-D (Table 2). It was soldered using the slanted incision-zigzag heating protocol. The center row represents welded incision in the cornea of piglet-G (Table 2). It was welded using the slanted incision-zigzag heating protocol. The bottom line represents the cornea of piglet-K (Table 2). This piglet belonged to the control group where a slanted incision was not laser heated.

The aim of the follow-up was the observation of the corneal shape. It was also safe to assume that after a recovery period of 6 weeks, the major contribution to P_B should have been attributed to the recovery, rather than to the protocol. Therefore, P_B was not tested on *in vivo* eyes.

The results are presented in Table 2 and summarized in Fig. 3. Three OCT findings and six histology findings are compared, among the piglet-eyes that were operated on. The OCT parameters are: (1) the observation of the slanted cut, (2) the extent of the hyperreflectivity on the anterior corneal side, and (3) the extent of the hyperreflectivity of the posterior stromal side. The histology parameters are: (1) the bonding quality, (2) the extent of epithelium thinning, (3) the folding of the basal-cell layer due to the heating, (4) the appearance of scarring, (5) the hypercellularity and neo-vascularization, and (6) the integrity of the Descemet's membrane. The first row in Table 2, describing the results for piglet, A, lists these findings, in a numbered order. The other piglets refer to these findings in the same order, with the appropriate changes.

In Fig. 3, we describe the typical OCT and histopathological results of three bonded incisions in three piglet corneas. Each cornea represented one of the three groups: soldered corneas, welded corneas, and a control group. Each incision is marked by two arrows.

These images show that the incisions from the epithelium to the endothelium (the two bold arrows marking the incision) were made at the edge of each cornea, near the limbus. They mark the entry and exit points at the front and back corneal surfaces (as opposed to depth, which is measured at a right angle to the corneal surface). The arrowheads mark the location of the disruption. This is the stromal scar from the epithelium to the endothelium, the dark line in the histological images, or the bright line in the OCT images.

3.2.1 *Soldered group*

The incision shown in Fig. 3 for piglet D was soldered using the slanted incision-zigzag heating protocol. A well-demarcated scar is clearly seen. This image shows good bonding, closed incision, and intact corneal shape, which indicates that no changes in corneal shape were caused by the soldering protocol.

The OCT analyses revealed slanted prolonged penetrating incisions, with hyperreflective anterior stroma, encompassing roughly the outer 40% to 70% of the incision length. A small area of the posterior stromal hyperreflectivity, near the endothelial side of the incision is seen. In one piglet, we could detect a prolonged area of the posterior stromal hyperreflectivity, near the endothelial side of the wound. In another piglet, we found an area of posterior stromal hyperreflectivity, near the endothelial side of the wound that bulged into the anterior chamber.

The histopathology analyses of the white-light microscopic images of the corneas in this group revealed well-bonded incisions in the corneal tissues. In the area of the bonded cut, the epithelium was mildly thinned, and the basal cell layer showed

Table 2 Experimental results for laser-soldering of piglet corneal-incisions, *in vivo*.

Action	Piglet	OCT	Histology
Group I solder: slanted incision, zigzag heating	A	1. Slanted prolonged penetrating cut. 2. Hyperreflective anterior corneal side, encompassing roughly the outer 40% to 70% of the wound length from the entry at the front corneal surface to the exit at the back corneal surface. 3. Small area of the posterior stromal hyperreflectivity near the endothelial side of the wound.	1. Well-bonded corneal cut. 2. Mildly thinned epithelium at the bonded cut. 3. The basal cell layer shows mild folding. 4. Thin epithelium-to-endothelium stromal scar. 5. The scar shows mild hypercellularity and neovascularization. 6. The Descemet's membrane is disrupted and the gap between its edges is occupied by a triangular scarring with the base toward the inner surface of the cornea.
	B	1. Same as the OCT results for piglet A. 2. Same as piglet A but only 50% of the wound is encompassed. 3. Prolonged posterior stromal hyperreflective area near the wound endothelium.	Same as the histology results for piglet A.
	C	1, 3. Same as the OCT results for piglet A. 2. 40%.	Same as the histology results for piglet A.
	D	1. Same as the OCT results for piglet A. 2. 70%. 3. Area of the posterior stromal hyperreflectivity near the endothelial side of the wound, bulging into the anterior chamber.	1, 2, 3, 4. Same as the histology for piglet A. 5. The scar shows mild hypercellularity and extensive neovascularization. 6. The Descemet's membrane is disrupted and is covered by endothelium.
Group-II weld: no albumin, slanted incision, zigzag heating	E	1, 2. Same as the OCT results for piglet D. 3. Minimal posterior stromal hyperreflectivity near the wound endothelium within some localized stromal thinning.	Same as the histology results for piglet D.
	F	1, 2, 3. Same as the OCT results for piglet D.	Same as histology results for piglet D.
	G	1, 3. Same as OCT results for piglet D. 2. 50% to 70%.	1, 2, 4, 5, 6. Similar to the histology for piglet A. 3. Not seen. Extensive inflammation is present at the cornea peripheral to the bonded perforated cut and limbus at this side.
	H	Experimental failure	Experimental failure

Table 2 (*Continued*).

Action	Piglet	OCT	Histology
Group-III control: slanted cut, no albumin, no laser heating.	I	1. Same as the OCT results for piglet D.	1, 2, 4, 5, 6. Similar to the histology for piglet A.
		2. 20% to 30%.	3. Not seen.
		3. Same as the OCT results for piglet D, with slight bulging into the anterior chamber.	There is adhesion between the inner surface of the scar and the anterior part of the iris.
	J	1, 2, 3. Same as the OCT results for piglet I,	Same as the histology results for piglet I.
	K	1, 2, 3. Same as the OCT results for piglet I.	Same as the histology results for piglet I.
		Massive excess hyperreflective tissue bulging into the anterior chamber, anterior to a separate defined anterior chamber structure, separated from the cornea by a thin membranous limit.	
	L	1, 2. Same as the OCT results for piglet I.	1, 2, 3, 4, 5. Similar to the histology for piglet A.
		3. Same as the OCT results for piglet D.	6. Same as the histology results for piglet D.

mild folding. A stromal thin scar was seen, extending from the epithelium to the endothelium with mild hypercellularity and microneovascularization (observed, but not shown). The Descemet's membrane was disrupted and the gap between its edges was occupied by a triangular-shaped scarring with the base toward the inner surface of the cornea.

3.2.2 *Welded group*

The histopathology and the OCT images shown in Fig. 3 for piglet G are those of an incision that was laser welded, using the slanted incision-zigzag heating protocol.

Most of the findings were similar to the ones of the soldered group (see above). Here, again, a completely closed incision is shown and the intact corneal shape indicates that the welding protocol did not cause a change in corneal shape. Some hyperreflectivity was noted on the outer layer of the cornea in the OCT images, which could be an indication of minor thermal damage. In the histopathology, a well-bonded incision was observed. In one sample, the cornea slightly bulged into the anterior chamber, and in another a possible minor wound sliding was detected.

3.2.3 *Control group*

The OCT image of the eye presented in Fig. 3 for piglet K (control group) shows an intact corneal shape that would indicate no detectable optical aberrations due to the protocol and a completely closed incision. Other findings were hyperreflectivity at the cut, some hyperreflectivity on the outer layer of the cornea, as well as some hyperreflective globular mass at the Descemet's membrane, even though no laser heating was applied. The histopathology image showed a well-sealed cut. A hyperreflective anterior stroma, encompassing roughly the outer 30% to 70% of the incision length, was seen, bulging into the anterior chamber. A measurable area of the posterior stromal hyperreflectivity near endothelial side of the wound was detected.

Figure 3 shows corneal images of three piglets, each representing one of the three groups. We notice that the radii of curvature of all the corneas, as determined by OCT and histopathology measurements, were similar in these groups. The results were similar to the ones observed in eyes extracted from pigs that were not processed in any way (including incising).

4 Discussion

Eyes that underwent corneal surgery must be able to withstand burst pressures that can be developed in various physiological responses, such as sneezing or coughing. As mentioned, the acceptable P_B for bonded corneal incisions is at least 80 mmHg.[36,37,41] The first aim of this work is to achieve this, or higher P_B, immediately after the surgery. Toward this goal, we tested different irradiation and incision protocols. These included the vertical incision-linear heating, the vertical incision-zigzag heating, and the slanted incision-zigzag heating protocols, as presented in Table 1.

It can be observed in Table 1 that in the *ex vivo* experiments the slanted incision-zigzag heating protocol generated nearly a 10-fold higher P_B than the generally acceptable one. This is probably because the spreading of the albumin on a larger area and because the laser heating was done on this larger area (on the surface and in the bulk). The vertical incision–linear heating and the vertical heating-zig zag protocols generated P_B that was still higher than the quoted minimally acceptable value of P_B.[36,37,41]

We assumed that the same P_B values will apply for the *in vivo* cases. It is important to notice that the incisions in the control group eventually closed since these incisions are self-sealing. However, as mentioned, with no laser bonding the P_B was only 30 mmHg, which is far too low. Therefore, only the soldered incisions achieved a water-tight seal of very high strength, immediately after surgery, whereas the welded or nonheated incisions did not.

Though a more thorough comparison may be needed, the bonding quality obtained with the 1.9-μm laser is better than the one obtained with a CO_2 laser.[29] This is most probably due to the difference in penetration depths and a more uniform heating along the depth of the incision.

We also note the relatively large standard deviation of the P_B results (Table 1). Our initial investigations traced this to how accurately the sample was cut. Introduction of "knifes" better than the standard scalpels may be needed. Femtosecond lasers were recently successfully adapted for ophthalmic laser surgery.[42] This advancement has the potential to increase the reproducibility in the P_B results.

The *in vivo* experiments were performed under the assumption that the same immediate P_B would be maintained if the irradiation protocol of the optimized *ex vivo* experiments is repeated. The aim in the *in vivo* experiments was not to observe P_B but to observe the corneal shape, after the weeks-long recovery period.

We believe that, as in any change in stromal integrity, there should be some change in the curvature of the cornea. The quantification of the degree of corneal distortion was not performed, using corneal topographic scans, as it was not available to us. We therefore attempted to qualitatively assess the change using both OCT and histopathology measurements (e.g., Fig. 3). We found that the histology and the OCT findings in corneas reported in Table 2 are quite similar for the three study groups. These showed complete closure and negligible change in corneal shape. From this, we conclude that the optical aberrations generated by this procedure should be negligible.

The OCT and histopathology measurements also revealed that the differences in the outcome were mostly in the area of the posterior stromal hyperreflectivity, near the endothelial side of either wound. In the soldered group it was small, whereas it slightly increased in the welded group and further slightly increased in the control group. Yet, all these changes were small. We note the overall similarity in all the images we obtained, regardless of the group to which they belonged. This similarity between the groups suggests that if there was thermal damage in the welding and soldering samples, it was insignificant.

We observed inflammation only in one *in vivo* eye out of the 12 quoted. This inflammatory reaction may be attributed to an abnormal recovery and not to the surgical protocol itself.

The outcome of this work is a demonstration that laser-soldering of corneas is feasible and that it generates an immediate and high burst-pressure bond. This should be of benefit to future corneal surgical protocols. Perpendicular incisions should be used for corneal transplantation, and we showed that the acceptable P_B has been achieved in this case. As for cataract surgery, it can benefit from the slanted incision-zigzag heating protocol, due to the exceptionally high bonding strength obtained with this improved method of corneal incision-closure.

Some of the advantages of laser soldering of corneal incisions, compared to suturing, include the following. (1) Immediate closure of nonpenetrating corneal wounds, such as in deep anterior lamellar keratoplasty. (2) Negligible optical aberrations and very little scarring were observed, thus demonstrating an optically transparent tissue. This will be beneficial for reducing postsurgical complications such as astigmatism. (3) In the case of corneal transplantation, a much easier and faster closure of a full 360-deg corneal graft has the potential to be obtained. (4) Reduction of the postoperative foreign body (sutures and stitches) sensation and discomfort.[43] (5) Sealing of miniature cuts that may appear in the cornea as a result of a trauma. Such cuts are extremely hard to close, so the surgeons are relying on the corneal self-healing while accepting the deformations that are generated in the curvature of the cornea as a result.

All these important advantages could make laser soldering a very promising technique for various types of corneal surgery.

5 Conclusions

Laser bonding of incisions was successfully demonstrated by several groups worldwide. However, it has not yet made a clinical impact.

We developed a fiber-optic temperature-controlled laser-soldering system for bonding corneal incisions, using albumin as a solder. The use of visible and mid-IR fibers made it possible to accurately monitor and control the temperature of each bonded spot on an incision and to do it rapidly. We used this system for bonding either vertical incisions or slanted incisions in corneas of piglets. A strong and watertight bonding was obtained immediately in both procedures. We did not observe a change in the corneal shape if the eye was soldered, welded, or left to self-heal.

The skill required to operate the laser soldering system is significantly lower than the one needed for suturing by an experienced surgeon. This is a noncontact method and a surgeon only needs to maintain the handpiece at a set distance above the cut and slowly move the distal end along the incision.

Future experiments will focus on corneal transplantation in large pigs, and the success in those experiments will enable us to start human clinical trials. We hope that in the future, laser soldering could substitute suturing in corneal surgery.

Disclosures

The authors have no conflicting interests to declare.

Acknowledgments

This work was funded by the Israeli Ministry of Science Grant Nos. 3-10856, 3-15627, and by the Pazi Foundation Grant No. ID22-2018.

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