Improved vascular tissue fusion using new light-activated surgical adhesive on a canine model

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Abstract. Newly developed light-activated surgical adhesives have been investigated as a substitute to traditional protein solders for vascular tissue fusion without the need for sutures. Canine femoral arteries ($n=14$), femoral veins ($n=14$), and carotid arteries ($n=10$) were exposed, and a 0.3-0.6 cm longitudinal incision was made in the vessel walls. The surgical adhesive, composed of a poly(L-lactic-co-glycolic acid) scaffold doped with the traditional protein solder mix of bovine serum albumin and indocyanine green dye, was used to close the incisions in conjunction with an 805 nm diode laser. Blood flow was restored to the vessels immediately after the procedure and the incision sites were checked for patency. The new adhesives were flexible enough to be wrapped around the vessels while their solid nature avoided the problems associated with ''runaway'' of the less viscous liquid protein solders widely used by researchers. Assessment parameters included measurement of the *ex vivo* intraluminal bursting pressure 1–2 h after surgery, as well as histology. The acute intraluminal bursting pressures were significantly higher in the laser-solder group (>300 mmHg) compared to the suture control group (<150 mmHg) where four evenly spaced sutures were used to repair the vessel ($n=4$). Histological analysis showed negligible evidence of collateral thermal damage to the underlying tissue in the laser-solder repair group. These initial results indicated that laser-assisted vascular repair using the new adhesives is safe, easy to perform, and contrary to conventional suturing, provides an immediate leak-free closure. In addition, the flexible and moldable nature of the new adhesives should allow them to be tailored to a wide range of tissue geometries, thus greatly improving the clinical applicability of laser-assisted tissue repair. © *2001 Society of Photo-Optical Instrumentation Engineers.*
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1 Introduction

The conventional method of performing vascular anastomoses is by suture repair.[1] Sutured anastomoses, however, can be time consuming, cause vessel narrowing, and lead to thrombosis at the site of the repair. The amount of suture inside the vessel wall can itself irritate the inside of the vessel, triggering thrombosis. Closely spaced sutures may cause tissue ischemia with resultant necrosis of the vessel margins. Other mechanical techniques currently used for vascular repair, including staples and clips, suffer from similar problems.[1] These factors have prompted many investigators to search for tissue adhesives that would ensure atraumatic tissue union. Among the various synthetic adhesives available, cyanoacrylates are the most popular.[2,3] However, they are toxic to the tissues, not absorbed in the normal wound healing process, and cause foreign body granulomas and allergic reactions. Fibrin glue has also been used as a biological surgical adhesive.[4] Fibrin glue imitates the final step of blood coagulation and, consequently, has been used effectively for hemostasis. However, repairs formed with fibrin glue alone are typically weak in comparison to suture repairs. Consequently, fibrin glue is almost always used in conjunction with a few stay sutures.[4] Finally, gelatin-resorcinol-formaldehyde/glutaraldehyde (GRF) glues are commonly used to assist repair of aortic dissections. When combined, the glue produces a waterproof lattice of macromolecules and has been found to display excellent adhesive properties.[5] However, unsatisfactory long-term complications, including heavy fibrosis and redissection at the site of glue application, have raised some concern about the use of GRF glues.[6]

Low strength anastomoses and thermal damage of tissue are major concerns of laser tissue welding techniques where laser energy is used to induce thermal changes in the molecular structure of tissues being joined, hence allowing them to bond together.[7] Current laser repair technology makes use of

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chromophore-enhanced protein solders.[8–16] Laser tissue soldering is a bonding technique in which protein solder is applied to the tissue surfaces to be joined, and laser energy is used to bond the solder to the tissue surfaces. The addition of protein solders to augment tissue repair procedures significantly reduces the problems of low strength and thermal damage associated with laser tissue welding techniques. Application of the solder to the repair site can be difficult, however. Liquid protein solders suffer from problems associated with ''runaway'' of the low viscosity material. Solid protein solders, while offering improved repair strength over liquid solders, are brittle and inflexible, and thus, not easily adapted to different tissue geometries.

The use of synthetic polymers as a scaffold for traditional protein solders provides a new line of surgical adhesives, which overcome many of the problems associated with previous laser tissue soldering techniques. The synthetic polymer membranes are porous and quickly absorb the traditional protein solder mix. The membranes are pliable and elastic and thus easily processed and fabricated into different forms and shapes. In addition, upon activation with a laser, the solder-doped polymer membranes tend to undergo a small amount of shrinkage. This shrinkage helps to maintain edge alignment as the tissue edges are gently pulled together. The membranes are also biodegradable. Foreign body reaction is expected to be minimal using the new adhesive, as the degradation products of the membranes, including lactic acid and glycolic acid, are naturally occurring substances inside the body.[17] The degradation rate of the membranes can be controlled by altering the macromolecular structure of the scaffold, that is the ratio of polylactic acid (PLA) to polyglycolic acid (PGA) of the poly(L-lactic-co-glycolic acid) (PLGA). Finally, the controlled release of various dopants including hemostatic and thrombogenic agents, antibiotics, anesthetics, and various growth factors can be added to the solder-doped polymer membranes to assist in the wound healing process.[7] Preliminary results from an *in vivo* study, conducted to assess these new solder-doped polymer membranes for laser-assisted vascular repair, are presented.

2 Materials and Methods

2.1 Preparation of Solder-Doped Polymer Membranes

Porous synthetic polymer membranes were prepared from PLGA with lactic:glycolic acid ratios of either 85:15 or 50:50 and poly(ethylene glycol) (PEG) with PLGA/PEG blend ratios of either 100:0 or 70:30, using a solvent-casting and particulate leaching technique.[18] One hundred milligrams of PLGA (Sigma Chemical Company, St. Louis, MO) and the required fraction of PEG (Sigma Chemical Company) were dissolved in 2 ml dichloromethane (Sigma Chemical Company), and combined with 70% weight fraction sodium chloride (salt particle size: 106–150 μm) to produce membranes with the desired level of porosity. The polymer was then cast into 60-mm-diam petri dishes and left in a fume hood for 24 h to allow the dichloromethane to evaporate. The salt was leached out of the polymer membranes by immersion in filtered de-ionized water for 24 h. During this period the water was changed 3–4 times. The membranes were then air dried and stored at room temperature until required.

Protein solder was prepared from either 25%(w/v) or 50%(w/v) bovine serum albumin (BSA) (Cohn Fraction V, Sigma Chemical Company) and indocyanine green (ICG) dye (Sigma Chemical Company) at a concentration of 0.5 mg/ml, mixed in de-ionized water. The solder was stored in light-proof plastic vials at 4 °C until required. Solder not used within one week was discarded.

The PLGA membranes used for incision repair were cut into rectangular pieces with approximate surface dimensions of 12×5 mm. The membranes were left to soak for a minimum of 2 h in the protein solder mix, before use. The average thickness of the solder-doped polymer membranes, determined by scanning electron microscopy (Philips 515, Holland) and measurement with precision calipers, was 202±12 μm.

2.2 Surgical Procedure

Seven adult mongrel dogs, weighing approximately 15 kg, were sedated with acepromazine maleate (10 mg/ml) then anesthetized with pentobarbital sodium (50 mg/ml) and endotracheally intubated. Hydration was maintained with a saline infusion (150–200 ml/h). After the groin was shaved, the superficial femoral arteries and veins were surgically isolated through bilateral groin/thigh incisions. Both the femoral artery and the vein were meticulously dissected from the surrounding tissues and separated from one another. Vessel diameters were 4–5 mm and 7–8 mm for the arteries and veins, respectively. The neck area was also shaved, a midline incision made overlying the trachea, and the carotid arteries exposed and isolated through blunt dissection into the carotid sheath. Vessel diameters were 5–6 mm. Before commencing vascular repairs, a small incision was made in the left femoral artery of each animal and a catheter containing a pressure transducer was inserted into the vessel. The catheter was advanced up the femoral artery for a distance of 15–20 cm, and the initial blood pressure of each animal was measured.

Prior to laser treatment the vessels were clamped both at the proximal and distal ends. A longitudinal incision about 0.3–0.6 cm long was made in the wall of the blood vessels. The vessels were rinsed with saline solution, and dabbed with a surgical swab to remove any residual blood. A strip of the solder-doped polymer membrane that did not contain PEG was then placed over the incision and laser irradiated. No stay sutures were used during this procedure. Upon completion of the weld, a second strip of the solder membrane, this time containing PEG, was applied on top of the first strip. The PEG gave the solder membrane a shrink wrap effect when heated with the diode laser. Hence, the solder membrane wrapped nicely around the first strip to complete the repair. Table 1 lists the experimental parameters used for each repair.

All laser-assisted anastomoses were created with a diode laser operating at a wavelength of 805 nm (Opto Power Corp., Tucson, AZ). The laser light was coupled into a 660-μm-diam silica fiber bundle and focused onto the membrane surface with an imaging hand piece connected at the end of the fiber. The diode was operated in continuous mode with a spot size at the membrane surface of approximately 2 mm. The laser system also incorporated an aiming beam, which was delivered through the same fiber as the 805 nm beam. An irradiance of approximately 15.9 W/cm^2, measured using a Molectron EPM 2000e power meter with a PM30 thermopile

Table 1 Experimental parameters used in the study.

Vessel	Solder membrane	Irradiation time
Femoral artery	Strip 1: 85:15 PLGA, 50% BSA	Strip 1: 100 s
($n=5$)	Strip 2: 85:15 PLGA, 30% PEG, 50% BSA	Strip 2: 80 s
Femoral artery	Strip 1: 50:50 PLGA, 50% BSA	Strip 1: 100 s
($n=7$)	Strip 2: 50:50 PLGA, 30% PEG, 50% BSA	Strip 2: 80 s
Femoral vein	Strip 1: 85:15 PLGA, 25% BSA	Strip 1: 80 s
($n=7$)	Strip 2: 85:15 PLGA, 30% PEG, 25% BSA	Strip 2: 80 s
Femoral vein	Strip 1: 50:50 PLGA, 25% BSA	Strip 1: 80 s
($n=7$)	Strip 2: 50:50 PLGA, 30% PEG, 25% BSA	Strip 2: 80 s
Carotid artery	Strip 1: 85:15 PLGA, 50% BSA	Strip 1: 100 s
($n=5$)	Strip 2: 85:15 PLGA, 30% PEG, 50% BSA	Strip 2: 100 s
Carotid artery	Strip 1: 50:50 PLGA, 50% BSA	Strip 1: 100 s
($n=5$)	Strip 2: 50:50 PLGA, 30% PEG, 50% BSA	Strip 2: 100 s

detector (Molectron Detector, Inc., Portland, OR), was delivered to the membrane surface. The laser beam was scanned in a continuous spiral pattern across the solder membranes two times (starting from the center). The total exposure time for each weld is listed in Table 1.

At the completion of the laser procedure the vascular clamps were sequentially released (arteries: distal then proximal; veins: proximal then distal) and flow was established. If an isolated leak point developed, vessels were reclamped and a second attempt was made to laser weld the region. The total time for solder placement and repairs was approximately 4 min for each anastomosis. Immediately following the laser procedure the vascular repairs were examined grossly for water tightness and patency was evaluated by visually observing vascular refill. After 1–2 h of flow, the animals were euthanized and the repaired section of the arteriovenous repairs explanted. Each specimen was then photographed and prepared for bursting pressure analysis.

A control study was performed *ex vivo* on excised femoral artery specimens ($n=4$). End-to-end anastomoses were performed with four interrupted 6-0 polypropylene sutures (United States Surgical Corp., Norwalk, CT), placed at equal intervals around the vessel wall. Upon completion of the repair, each specimen was prepared for bursting pressure analysis. Throughout the study, animal care complied with the ''Guide for the Care and Use of Laboratory Animals'' (NIH Publication No. 6-23, revised 1985).

2.3 Bursting Pressure Analysis

Bursting strength was determined by infusion of saline solution. A 50 ml syringe, filled with saline, was inserted into one end of the vessel and secured using a size 4-0 suture. A pressure transducer, attached to an amplifier, was inserted into the other end of the vessel, and likewise secured with a 4-0 suture. Bursting pressure data were acquired using a laptop computer containing a data acquisition card and BioBench software (National Instruments, Inc., Austin, TX). The vessel was deemed to have ''burst'' when saline was first observed to leak through the repair. Figure 1 shows a photograph of the experimental setup used for the bursting pressure analysis.

2.4 Histological Examination

Light microscopy was used to assess the histological characteristics of laser-induced thermal damage of the repaired specimens. Dissected specimens were fixed in formalin immediately after the bursting pressure measurements were made and stored at 6 °C until they could be prepared for staining and mounting. Masson's trichrome and hematoxylin and eosin were used as the staining agents.

3 Results

Examples of repairs formed using the solder-doped membranes are shown in Figures 2 and 3. After weld completion, unclamping of the vessel resulted in single focal leak points
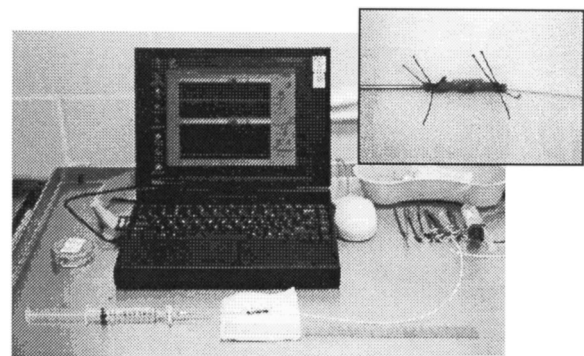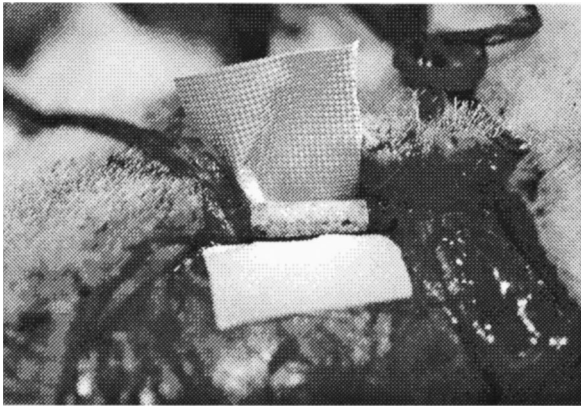

Fig. 1 Bursting pressure apparatus.

Fig. 2 Canine right carotid artery repaired using two solder-doped polymer membranes. The polymer membrane was formed using PLGA with a 85:15 copolymer ratio and 70% NaCl with particle sizes of 106–150 μm. PEG was added to the second membrane applied to the vessel. Each membrane was doped with protein solder composed of 50% BSA, 0.5 mg/ml ICG, and de-ionized water. Laser treatment parameters are listed in Table 1.

Fig. 4 Results of bursting pressure measurements made on excised carotid arteries ($n=10$), femoral arteries ($n=14$), and femoral veins ($n=14$), successfully repaired with the solder-doped polymer membranes. Table 1 lists the breakdown of experimental parameters used in this study.

that developed under arterial pressure in 8 of the 34 anastomoses. Laser rewelding was successful in sealing these anastomotic leaks. Thirty of the 34 laser-welded vascular repairs remained patent with high flows and palpable thrill during this acute experiment.

Results of bursting pressure measurements made on excised vessels successfully repaired with the solder-doped polymer membranes are presented in Figure 4. Altering the PLGA copolymer ratio appeared to have little effect on the strength of the repairs. In addition, no significant difference ($<2\%$) was observed between the strength of repairs formed using polymer membranes doped with 25% BSA and those doped with 50% BSA. Consequently, the graph of Figure 4 has been divided solely according to tissue type. The bursting pressure of the suture control group ($n=4$) was measured to be <150 mmHg.

Histological examination of the repair sites showed that the amount of thermal damage to the underlying tissue was minimal using the solder-doped polymer membranes. Typical examples of photomicrographs taken of the repaired tissue specimens are presented in Figures 5 and 6.

4 Discussion

Solder-doped polymer membranes have been developed as an alternative to conventional fasteners and traditional protein solders for tissue repair. The new membranes offer flexibility along with improved repair strength over previous published results using albumin protein solders.[14–16] The properties of the solder-doped membranes can be easily tailored to meet the specific requirements of a wide range of clinical applications. Both the albumin protein solder and the polymer membranes are biodegradable, and thus, foreign body reaction and infection are expected to be minimal. In fact, PLGA is commercially available, and already has Food and Drug Administration approval for clinical procedures. The new membranes
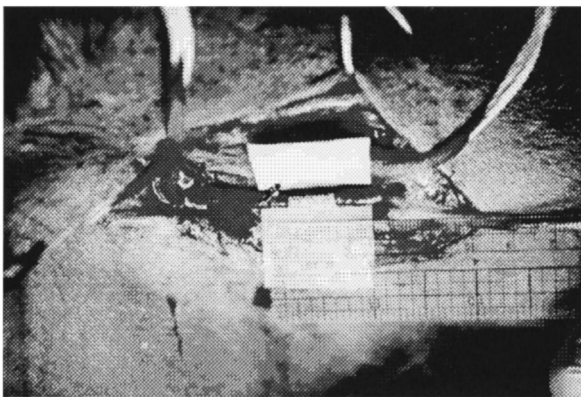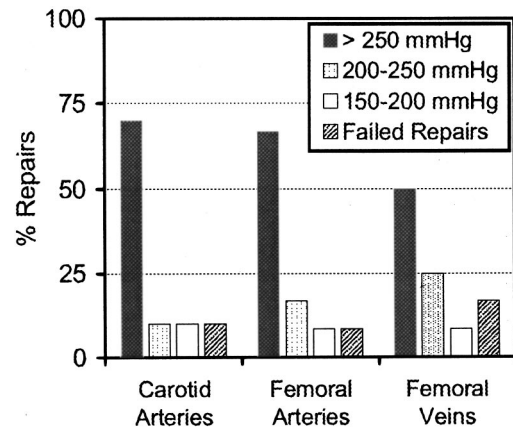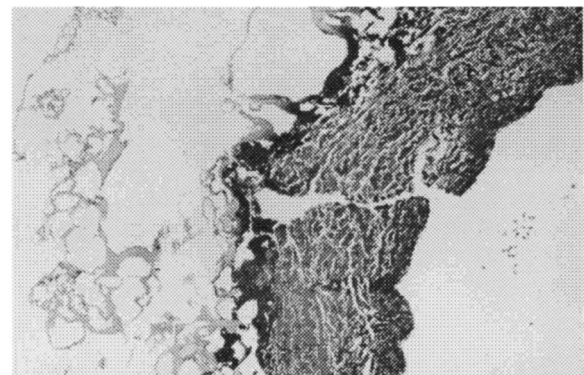

Fig. 3 Canine left femoral artery repaired using two solder-doped polymer membranes. The polymer membrane was formed using PLGA with a 85:15 copolymer ratio and 70% NaCl with particle sizes of 106–150 μm. PEG was added to the second membrane applied to the vessel. Each membrane was doped with protein solder composed of 50% BSA, 0.5 mg/ml ICG, and de-ionized water. Laser treatment parameters are listed in Table 1.

Fig. 5 Photomicrograph of canine left femoral vein repaired using solder-doped polymer membranes containing PLGA with a lactic:glycolic acid ratio of 85:15 (Mag: 40$\times$). Masson's trichrome was used as the stain. Minimal thermal damage to a depth less than 40 μm was seen in the tissue. Treatment parameters are listed in Table 1.
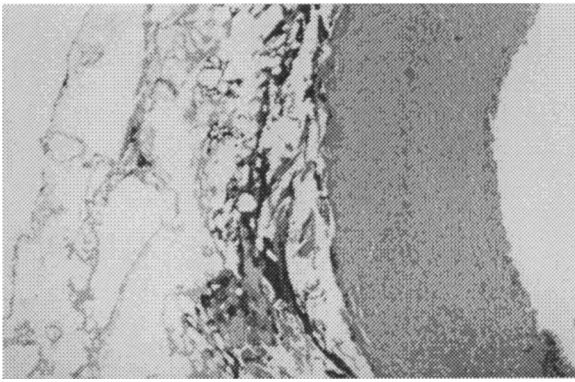
Fig. 6 Photomicrograph of canine right carotid artery repaired using solder-doped polymer membranes containing PLGA with a lactic:glycolic acid ratio of 85:15. Masson's trichrome was used as the stain. While the incision site is not shown in the micrograph, evidence of thermal damage at the treatment site is minimal (<25 μm). Treatment parameters are listed in Table 1.

provide a quick and easy method of joining tissue in surgery. Slight rehydration of the solder-doped polymer membranes upon application to the tissue assists with tissue apposition, thus relieving the need for stay sutures, often associated with laser tissue repair techniques.

Altering the PLGA copolymer ratio appeared to have little effect on the strength of the repairs. This observation supported the findings of our earlier *in vitro* studies using the solder membranes.[19] Variation of the PLGA copolymer ratio can, however, influence the membrane degradation rate.[17,18] In addition, no significant difference was observed between the strength of repairs formed using polymer membranes doped with 25% BSA and those doped with 50% BSA. This observation was in contrast with previous tissue soldering observations, which showed that the tensile strength of repairs was improved with the use of increased protein concentrations.[14,15,20] In a previous study, scanning electron microscopy analysis suggested that the 25% BSA solder was better able to permeate the polymer membrane.[21] Finally, the addition of PEG during the film casting stage increased the flexibility of the membranes. In this study, PEG was not contained in the membranes used during the first application of the adhesive. PEG tends to wash out of the polymer scaffolds during the salt leaching stage of their preparation. This leaves tiny pores in the scaffold, which are too small to absorb the traditional protein solder mix.[21] When membranes containing the PLGA/PEG composite were applied to a blood vessel during the first application of the adhesive, the blood was able to pass through the open pores, causing the vessel to leak. Membranes containing the PLGA/PEG composite, however, were found to provide the beneficial feature of a ''shrink wrap'' effect when used during the second application of the adhesive.

Future research will look at tailoring the properties of the membranes to meet the specific requirements of a range of clinical applications. The mechanical properties of the membranes are greatly influenced by the initial salt weight fraction and salt particle size. Increasing the salt weight fraction increases the porosity of the polymer membranes, and consequently, the stiffness of the membranes can also be signifi-

cantly reduced. Changing the salt particle size has less effect on the strength of the membranes,[17] however, the ability of the solder to permeate the membranes is a concern with lower salt particle size diameters. In addition, alternative biodegradable polymer substrates are to be investigated including PLA, PGA, and PLGA/PEG blends, as well as alternative chromophores including carbon black. Finally, development of new molds to create set membrane geometries suitable to various clinical applications, such as a T-mold for end-to-side vascular anastomosis, are being investigated.

5 Conclusions

The clinical feasibility of laser tissue repair using solder-doped polymer membranes has been demonstrated with respect to repair strength, repair stability over a 2 h period, and the development of a protocol for surgical application. Assessment of long-term anastomotic strength and stability and histological evaluation of the immunological response will be the objectives of a subsequent *in vivo* survival study. In the future, it could be possible to use patches prepared from the solder-doped polymer membranes in the field as a simple and effective method to stop bleeding and repair tissue quickly in an emergency situation.

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