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Surgical adhesives for laser-assisted wound closure

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Abstract. Solid protein solder-doped polymer membranes were developed for laser-assisted tissue repair. Biodegradable polymer membranes of controlled porosity were fabricated with poly(*L*-lactic-co-glycolic acid) (PLGA), poly(ethylene glycol) (PEG), and salt particles, using a solvent-casting and particulate-leaching technique. The membranes provided a porous scaffold that readily absorbed the traditional protein solder composed of serum albumin, indocyanine green dye, and de-ionized water. *In vitro* investigations were conducted to assess the influence of various processing parameters on the strength of tissue repairs formed using the new membranes. These parameters included PLGA copolymer and PLGA/PEG blend ratios, membrane pore size, initial albumin weight fraction, and laser irradiance used to denature the solder. Altering the PLGA copolymer ratio had little effect on repair strength, however such variations are known to influence the degradation rate of the membranes. The repair strength increased with increased membrane pore size and bovine serum albumin concentration. The addition of PEG during the membrane casting stage increased the flexibility of the membranes but not necessarily the repair strength. Typically, the repair strength increased with increasing irradiance from 12 to 18 W/cm^2. The new solder-doped polymer membranes provided all of the benefits associated with solid protein solders including high repair strength and improved edge coaptation. In addition, the flexible, moldable nature of the new membranes offers the capability of tailoring the membranes to a wide range of clinically relevant geometries. [DOI: 10.1117/1.1412436]

Keywords: poly(*L*-lactic-co-glycolic acid) (PLGA); albumin protein solder; indocyanine green dye; diode laser; laser-tissue welding.

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1 Introduction

Laser-tissue welding techniques have proven advantages over conventional mechanical wound closure techniques when suturing or stapling techniques are difficult or impossible to perform. These advantages include reduced suture and needle trauma,[1] reduced foreign body response,[2] reduced bleeding with immediate liquid-tight anastomosis intraoperatively, faster healing, and the potential for simpler methods of minimally invasive and endoscopic wound closure.[3] Despite these obvious advantages and the extensive research completed in this area, laser-assisted tissue repair has yet to gain widespread clinical acceptance. Experiments have shown unreliable fusion strength,[4–6] poor repeatability, thermal damage to the tissue,[1] and technical difficulties associated with tissue apposition. The addition of endogenous and exogenous materials to be used as solders and the use of laser wavelength-specific choromophores have improved the weld strength and repeatability of laser repairs while reducing tissue thermal damage. The combination of serum albumin and indocyanine green dye (ICG) has shown promising results.[3,7–17] The albumin solder is denatured at the repair site during laser welding to enhance the adhesion of the adjacent tissue edges. The resulting denatured solder is nonimmunogenic[14] and is gradually absorbed in the normal wound healing process.[12,13] Moreover, the presence of the solder coagulum increases the initial laser weld strength.[3] ICG has a maximum absorption coefficient at 805 nm of 2×10^5 mg^{-1} cm^{-1} and binds preferentially with serum protein,[18] ensuring that heat is efficiently transferred to denature the protein solder. Since tissue absorption at this wavelength is minimal, the amount of collateral thermal damage to the tissue by direct laser light absorption is minimized.

Use of a biodegradable polymer scaffolding to hold the solder during laser repair is an emerging technique shown to improve the success rate and repeatability of the solder tissue welds.[7] Solid protein solder-doped membranes eliminate ''runaway'' associated with less viscous, free running liquid solders. The solder-doped polymer membranes are also less brittle and more flexible than solid solder-only strips. This technique is ideal for clinical applications requiring the solder material to be modeled or shaped into various geometries.

The aim of this study was to determine the optimal parameters for laser-assisted wound closure using a surgical adhesive consisting of a polymer membrane doped with solid pro-

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tein solder. An *in vitro* study was performed to investigate the influence of various processing parameters on the strength of tissue repairs made with the new adhesives denatured by an 805 nm diode laser at a range of laser irradiances.

2 Materials and Methods

2.1 Surgical Adhesive Preparation

Liquid protein solder solution was prepared from bovine serum albumin (BSA) (Sigma Chemical Co.) (25% and 50%) and ICG dye (Becton Dickinson) (0.25 mg/mL) that was mixed in de-ionized water. The protein solder was stored in a lightproof vial at 4°C until needed. Solution remaining after 10 days was discarded.

Biodegradable polymer membranes were fabricated with poly(*L*-lactic-co-glycolic acid) (PLGA) (Sigma Chemical Co.) (85:15 and 50:50 copolymer ratio) and poly(ethylene glycol) (PEG) (Sigma Chemical Co.) (PLGA/PEG blend ratios of 100:0 and 70:30) using solvent-casting and particulate-leaching techniques. Dry PLGA and PEG powders were dissolved in dichloromethane (Sigma Chemical Co.) and combined with sodium chloride ($>$106 and 106–150 μm, 70 wt %) to control the size and number of pores in the final membrane. The mixtures were cast in covered 60 mm Petri dishes and left overnight in a fume hood to allow the dichloromethane to evaporate. The following day, the membranes were submersed into filtered, de-ionized water to leach the salt particles from the polymer, resulting in a porous polymer membrane. The membranes were left to soak in the water for 12 h. During this period, the water was changed four to five times. The membranes were then air dried and cut into rectangular strips having nominal dimensions of 1.5×5 mm^2.

Membrane strips were doped by soaking them in the liquid solder mixture for 2 h. By capillary action, the solder was drawn into the membrane pores. The strips were removed from the excess solder and allowed to dry on pieces of polyfilm. Dried strips were stored in lightproof glass vials at room temperature until needed. The resulting thickness of the solder-doped polymer membranes, determined by measurement with precision calipers, was 140 ± 6 μm. Strips that remained after 10 days were discarded.

2.2 Tissue Preparation

Bovine thoracic aortas were obtained from a local slaughterhouse (Taylor Meat Company, Taylor, TX). Fat and excess adventitial tissue were removed to obtain an aorta thickness of approximately 1 mm. The aortas were soaked in phosphate buffered saline (0.01 M phosphate buffer, 0.137 M sodium chloride, *p*H 7.4) (Sigma Chemical Company) for 15 min to remove blood from the tissue surface. The aortas were then wrapped in saline soaked gauze and stored at -20°C until required.[19] Unused tissue was discarded after 7 days.

2.3 Laser System

A GaAlAs semiconductor laser diode with a nominal output power of 5 W and wavelength of 805 nm (Opto Power Corp.) was used to denature the protein solder. The laser radiation was focused through a series of optical polarizers onto the specimen, which was held at a fixed position. The diode was operated in continuous mode with a spot size at the tissue surface of 1.5 mm. Losses through the optical polarizers reduced the laser power by 77%. Diode powers of 212, 265, and 318 mW, calibrated with a power meter (Molectron EPM 2000e) and a thermopile detector (Molectron PM30), were delivered to the surgical adhesive surface resulting in irradiances of approximately 12, 15, and 18 W/cm^2, respectively. A translation stage (Oriel Corp.) moved the specimen under the beam at a scanning speed of 0.3 mm/s. Each scan went approximately 0.5 mm beyond the end of the strip. Total irradiation time for four passes was 80 s.

2.4 Surgical Procedure

Before use, the aortas were thawed and cut into rectangular specimens with approximate dimensions of 2×1 cm^2. The intima side of the aorta was used as the repair surface. A full thickness incision was cut through the specimen width using a scalpel and opposing ends were placed together. The strip of adhesive was placed perpendicularly across the junction of the served aorta specimen. A weld was created by denaturing the protein solder with four passes of the diode laser output.

2.5 Strength/Breaking Load Analysis

Strength testing was performed on each specimen after the incision was repaired. The specimen was mounted horizontally in the grips of a gravity based weld strength-measuring device.[16] On one end, the specimen grip was fixed, while the other end was free to slide in a track. A cup was suspended from the free end and water was added to the cup at a constant rate of 450 mL/min. When the repair failed, the two halves of the tissue separated and the flow of water was stopped. The force required to break the repair was computed using the total mass of the cup plus the water (the mass that was needed to overcome friction in the sliding mechanism and to initiate motion of the free end of the specimen grips was measured for each specimen and subtracted from the breaking mass). Ten specimens were tested for each combination of membrane composition (PLGA copolymer ratio, PLGA/PEG blend ratio), membrane pore size, albumin weight fraction, and laser irradiance used to denature the solder. A total of 480 specimens were repaired and tested during this study.

3 Results

The breaking load of the tissue repairs as a function of adhesive processing parameters and laser irradiance is shown in Figures 1–4. The small and large membrane pores refer to membranes fabricated using $<$106 and 106–150 μm salt particle sizes, respectively. Figures 1 and 2 show the results of experiments conducted on PLGA only membranes at the two copolymer ratios investigated, 85:15 and 50:50, respectively. Figures 3 and 4 show the results of experiments conducted on PLGA/PEG membranes (70:30) at the two PLGA copolymer ratios investigated. The breaking load and standard deviation for each value of laser irradiance and adhesive parameters were determined from an average of 10 repairs. Extreme mathematical outliers were calculated using the box and whisker method, and were removed from the mean and standard deviation calculations.

The dehiscence rate was 1.7% for adhesives containing 25% BSA. The dehiscence rate was 5.0% for adhesives containing 50% BSA. Two different failure mechanisms were observed in the tissue repairs: cohesive failure and interfacial

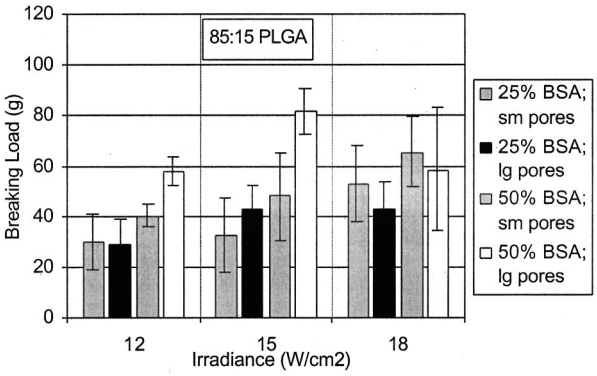

Fig. 1 Breaking load of tissue repairs formed using PLGA only membranes with 85:15 copolymer ratio.

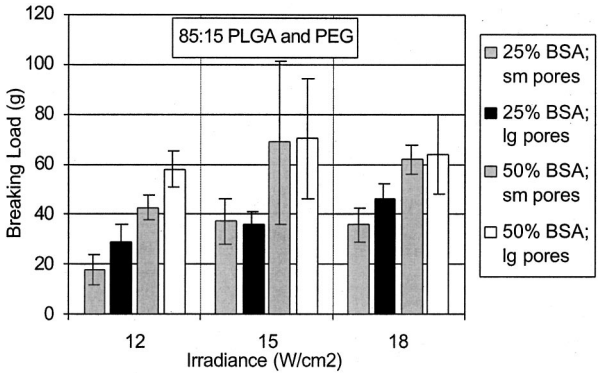

Fig. 3 Breaking load of tissue repairs formed using 70% PLGA and 30% PEG membranes with a 85:15 PLGA copolymer ratio.

failure. These terms are based upon Comyn's representation of single lap joint fracture modes.[20] Cohesive failures occur when the adhesive strip breaks into two halves, but each remains attached to the tissue. Interfacial failures occur when the strip remains intact but detaches from the tissue. Only 3.5% of the failures were cohesive. The strength of the cohesive failures was typically lower than that of the interfacial failures, however there are a few distinct cases in which the strength of the cohesive failures were significantly higher than the interfacial failures. Due to the dominant breaking mechanism being that of interfacial failures, breaking load measurements are more appropriate for comparing the various adhesive parameters than tensile strength calculations. Finally, the breaking strength of the adhesive alone, without tissue or laser irradiation, was significantly higher (218.4 ± 15.6 g) than that of the tissue repairs formed using the adhesive (81.6 ± 8.6 g).

Several trends are evident from Figures 1–4.

(i) The strength of the tissue repairs generally increased with increased irradiance for the values tested. For one case (85:15 PLGA with 50% BSA and large pores in Figure 1) increasing the intensity from 15 to 18 W/cm^2 lowered the strength of the repair as statistically proven with a 0.05 level of significance Student T test.

(ii) Increasing the BSA concentration from 25% to 50% improved repair strength.

(iii) Increasing the pore size of the membranes generally improved the repair strength except for one case, 85:15 PLGA, 25% BSA adhesives irradiated at 18% W/cm^2 shown in Figure 1.

(iv) In general, the addition of PEG to the initial polymer mixture did not affect the final repair strength. Student T tests show the strengths were statistically the same for 21 of the 24 different comparisons. The tree variant comparisons had decreased repair strength when 30 wt % PEG was added to adhesives consisting of 85:15 PLGA, large pore membranes, and 25% BSA solder when irradiated with 12 or 18 W/cm^2, as shown in Figure 1.

The maximum breaking load achieved for tissue repairs using laser-activated surgical adhesive was 81.6 ± 8.6 g for the 106–150 μm large pore, 85:15 PLGA only membranes doped with 50% BSA solder irradiated at 15 W/cm^2.

The strength of repairs formed using the lower irradiance of 12 W/cm^2 was not significantly better than that formed using traditional liquid (25% BSA) protein solders without the polymer membrane as a backbone [34.8 ± 3.8 g (refer to Table 1)]. While the repair strength improved with increased irradiance, the strength of repairs formed using irradiances of 15

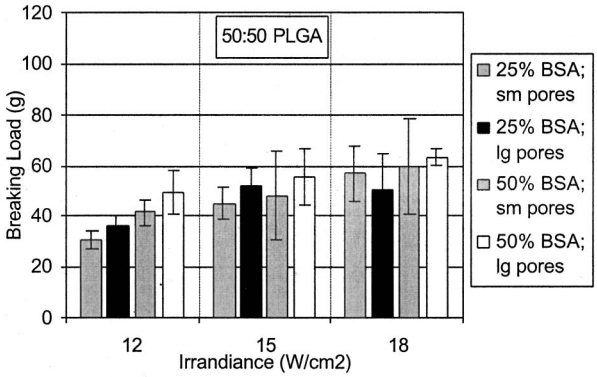

Fig. 2 Breaking load of tissue repairs formed using PLGA only membranes with a 50:50 copolymer ratio.

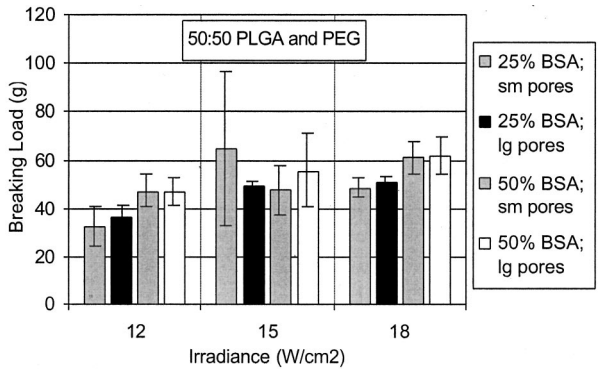

Fig. 4 Breaking load of tissue repairs formed using 70% PLGA and 30% PEG membranes with a 50:50 PLGA copolymer ratio.

Table 1 Breaking strength of the native aorta and strongest repairs formed using liquid solder (Refs. 21 and 22), solid solder (Refs. 21 and 22), and solder-doped membranes.

Solder type	Irradiance (W/cm^2)	Breaking strength (g)
Liquid solder (25% BSA)	12.7	34.8±3.8
Solid solder (60% BSA)	6.4	91.5±4.2
Solder-doped membrane (50% BSA, 85:15 PLGA)	15.0	81.6±8.6
Native tissue	···	89.4±4.7

W/cm^2 to activate the adhesives was still inferior to that achieved using traditional solid (60% BSA) protein solders [91.5±4.2 g (refer to Table 1)]. However, the strength of repairs formed using the adhesives denatured at 15 W/cm^2 compared favorably with the strength of the native aorta [89.4 ±4.7 g (refer to Table 1)].

4 Discussion

The irradiance of 15 W/cm^2 for the maximum strength is much higher than previously reported for liquid or solid solders.[17,21,22] This increase is believed to be due to scattering of the laser light in the adhesive and the packing density of the solder.[7] Sacrificing some of the tissue repair strength for increased adhesive control and flexibility can be advantageous in surgical procedures with irregular geometries. Additionally, maximum repair strength may not be the ideal procedure end point because it may be associated with an unacceptable amount of thermal injury. Surgeons generally look for visible signs of the tissue being denatured, such as discoloration, as markers of the end point of welding procedures. This is a highly subjective and qualitative indicator. In this study, bonds formed at irradiances where no color change was observed. Perhaps visual indicators of tissue denaturation should be replaced with the use of predetermined irradiance and exposure times. This is feasible with these surgical adhesives because of their uniform characteristics.[23]

PLGA was selected for the biodegradable membrane because this hydrophilic polymer and its individual constituents (polylactic acid and polyglycolic acid) are commercially available, and are already FDA approved for surgical procedures. These polymers gradually degrade into lactic acid and glycolic acid which are found in the human body. However, these acids will lower the *p*H of the local cell environment. Semicrystalline L-poly(lactic acid) was selected for increased mechanical strength and toughness.

For most combinations, altering the PLGA copolymer ratio had little effect on repair strength, however such variations are known to influence the degradation rate of the adhesives. The copolymer has a significantly higher degradation rate than the individual polymers due to the alternating chain structure that reduces crystallinity. The 50:50 copolymer ratio will degrade faster than the 85:15 ratios and can be easily altered to match the rate of tissue regeneration *in vivo*. The porous structure of the polymer membrane will further increase the degradation rate due to the high surface area to volume ratio. The porous adhesive will support cell differentiation by mimicking the extra cellular matrix of tissue. Additionally, larger membrane pores result from the use of larger salt particles during fabrication of the membrane. Larger pores allow more solder to penetrate the membrane. The increase in solder may be the reason for the stronger repairs noted with adhesives processed with the larger salt particles.

In general, no statistical significant difference was observed for the adhesives fabricated with PEG determined using a student T test with a 0.05 level of significance. However, the rationale for the addition of PEG was not to improve the adhesive strength, but to improve the material flexibility and possibly decrease *in vivo* immunological responses. Due to PEG's water solubility, most of the PEG is leached from the membrane when submersed in water and leaves micropores.[24,25] The micropores improve the membrane flexibility needed for *in vivo* applications with unique geometries. Additionally, it is well documented that modifying material surfaces with PEG can suppress or eliminate protein absorption and platelet adhesion while improving interfacial chemistry.[26,27] Thus, the small amount of PEG remaining in the adhesive may improve biostability and synergistic blood compatible effects.[28,29]

Finally, increasing the BSA concentration of the solder from 25% to 50% increased the repair strength of the adhesives (Figures 1–4). This observation supports the results of previous tissue soldering experiments, which showed that the tensile strength of repairs was improved with the use of increased protein concentrations.[21,22]

5 Conclusion

The features of the new laser-activated surgical adhesives presented could greatly improve the future of clinical applications of laser–solder wound closure. The properties of the adhesives can be easily tailored to meet the specific requirements of a range of clinical applications including vascular anastomoses, urethral reconstructions, and spleen and liver lacerations. The reinforcing materials might additionally serve as carriers for antibiotics, growth factors, or other drugs to aid in healing. In the future, it may be possible to use large patches of this adhesive in the field as a simple and effective method by which to stop bleeding and repair wounds quickly in emergency situations. The results obtained in this *in vitro* study are encouraging for the future of laser-assisted tissue repair. Further studies in an *in vivo* setting will be required to evaluate the postoperative recovery rate, the effects of the laser and adhesive on surrounding tissue, neovascularization, and tension on the repair site.

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