Laparoscopic Mesh Fixation Using Laser-Assisted Tissue Soldering in a Porcine Model

Raymond J. Lanzafame, MD, MBA, Barbara A. Soltz, PhD, Istvan Stadler, PhD, Robert Soltz, BS

ABSTRACT

Background and Objective: Animal studies using open surgical models indicate that collagen solder is capable of fixation of surgical meshes without interfering with tissue integration, increasing adhesions, or increasing inflammation intraperitoneally. This study describes development of instrumentation and techniques for laparoscopic herniorrhaphy using laser-assisted soldering technology.

Study Design and Methods: Anesthetized 20 kg to 25 kg female Yorkshire pigs underwent laparoscopy with a 3-trocar technique. Parietex TET, Parietex TEC, and Prolene mesh segments (5 x 5 cm) were embedded in 55% collagen solder. Segments were inserted by using a specially designed introducer and affixed to the peritoneum by using prototype laser devices (1.45 μ, 4.5 W continuous wave, 5-mm spot, 55° C set temperature) and a custom laparoscopic handpiece (IPOM). Parietex PCO mesh was inserted and affixed using the Endo-hernia stapler (Control). Animals were recovered and underwent second-look laparoscopy at 6 weeks. Mesh sites were harvested after animals were euthanized.

Results: The mesh-solder constructs were easily inserted and affixed in an IPOM approach. Prolene mesh tended to curl at its edges as the solder was melted. Postoperative healing was similar to that in Control segments in all cases.

Discussion and Conclusion: Collagen-based tissue soldering permits normal wound healing and may mitigate or reduce the use of staples or other foreign bodies for laparoscopic mesh fixation, prevent tissue ischemia and possibly nerve entrapment, which result in severe postoperative pain and morbidity. Laser-assisted mesh fixation is a promising alternative for laparoscopic herniorrhaphy. Further development of this strategy is warranted.

Key Words: Collagen, Experimental surgery, Hernia repair, Laparoscopy, Laser surgery, Peritoneum, Tissue solder, Wound healing.

INTRODUCTION

Herniorrhaphy is a common surgical procedure. Prosthetic mesh is typically used for tension-free reinforcement of the defect in the abdominal wall in both open and laparoscopic herniorrhaphy techniques. Mesh reinforcement provides for stabilization of the abdominal wall, serves as a replacement for thinned or absent native tissues, and restores mechanical functionality. Mesh requires fixation to the site of the hernia defect, and the ultimate bioincorporation of the material accounts for the integrity of the repair, reduces the potential for mesh migration, and influences outcomes including short- and long-term complications.

Various mesh fixation strategies have been used clinically. Mechanical methods for mesh fixation often result in local tissue ischemia, can cause nerve entrapment, severe local pain, and could be a nidus for adhesion formation in intraperitoneal approaches. The use of surgical glues for mesh fixation and wound closure has been reported. However, the use of tissue adhesives and various materials to coat or cover the intraperitoneal surface of mesh can have a significant influence on tissue ingrowth and the tensile strength of the resultant repair.

Previous work from our laboratory demonstrates that temperature controlled laser collagen soldering results in satisfactory acute tensile strengths after mesh fixation and demonstrates that adhesion formation is reduced in scenarios that cover the mesh material with the collagen solder. Bioincorporation of the mesh-solder constructs is histologically similar to mesh with staple fixation in these models. The present study was undertaken to investigate tissue histology and bioincorporation of mesh...
materials following the use of derivatized collagen solder for intraperitoneal mesh fixation during laparoscopic herniorrhaphy. Instrumentation and techniques for laparoscopic herniorrhaphy using laser-assisted soldering technology and various mesh-solder constructs were evaluated in a porcine model.

**MATERIALS AND METHODS**

**Solder Preparation**

Preparation of collagen solder films has been described previously. Briefly, the collagen for all solder formulations was prepared from porcine or calf corium. Purified, telopeptide-poor Type I collagen was derivatized with glutaric anhydride. The anhydride reacts with deprotonated free amines and substitutes a carboxyl group for the reacted amine group, making the composition anionic. The degree of derivatization was selected so that the modified collagen remains soluble at physiologic pH. Derivatization was performed by adjusting the pH of soluble collagen (5 mg/mL) to 9.0, using NaOH, adding solid anhydride to the collagen in the range of 10% to 30% (w/w) solution and maintaining the pH at 9.0 during the reaction. After 15 minutes, the pH of the solution was reduced to about 4.5 to precipitate derivatized collagen. The precipitate was recovered by centrifugation at 14500 RPM for 20 minutes and at 9°C. The precipitate was washed 2 times with sterile water. The final precipitate was dissolved in 5mM phosphate buffer at pH 7.2 at a final concentration of 5 mg/mL. The solution was freeze dried in trays at a controlled rate. Solder films were prepared from the lyophilized stock collagen. Lyophilized sheets were cut into small pieces and homogenized in a tissue mill (IKA A11; IKA Works, Inc, Wilmington, NC). Films were prepared by dissolving collagen powder in distilled, sterile water. The collagen solid concentration ranged from 30% to 50% and was obtained by exposing the dispersions to a controlled temperature water bath. As the collagen dissolved, more was added until the desired weight-to-volume concentration was achieved. The viscous solution was centrifuged with mesh segments and poured into molds. A Teflon plate was pressed onto the filled mold while the mixture was still warm to control film thickness. Parietex TET, Parietex TEC (Covidien, Inc, Mansfield, MA), and Prolene (Ethicon, Inc., Somerville, NJ) mesh segments (5 x 5 cm) were embedded in 55% collagen solder. The mesh-solder pledgets were removed from the mold after cooling for 3 minutes, vacuum packaged, labeled, and stored at 4°C until sterilization. The prepared packaged mesh solder films were subsequently sterilized by E-beam prior to their use.

**Laser System and Delivery Devices**

The laser system consists of a fiber laser, controller board for the sensor, switches, displays, indicator lamps, interfaces, calibration hardware and an ambient air-cooling fan. This system operates in continuous wave (CW) mode and consists of a controller assembly, computer circuitry, interconnect wiring, and a handpiece. Optimal laser parameters, such as wavelength and power, were selected based on earlier animal experiments. Devices operating at a wavelength of 1.533 μ and 4.8 W continuous power level (CW) or at a wavelength of 1.45 μ and at 4.5 W CW, with a 5-mm beam spot and a 55°C set temperature were constructed for this investigation. The laser output is controlled by a foot-switch. The laser output level is determined by a circuit that compares the set temperature with the measured temperature from an infrared sensor that is contained in the laparoscopic handpiece.

The laser is coupled to a fiber 1mm in diameter. The laser controller consists of a detector that senses radiation from the heated weld site and outputs a signal related to the intensity of the temperature at this site. There is circuitry that conditions the detector signal and a computer that is programmed with algorithms that compute the weld site temperature for comparison with a set temperature as well as determining the laser input control signal that ultimately drives the laser output. This control loop (ie, temperature sensing, set temperature comparison and output to control the laser to increase or decrease power output) continuously monitors temperature and laser modulation to approach and maintain the set temperature. Two optical fibers (one to collect and transmit blackbody radiation in the 7 μ to 15 μ range to the detector and the other to transmit laser energy to the weld site) are aligned and secured in a delivery device that is held by the surgeon. The handpiece is geometrically configured to ensure that the detector fiber is collecting radiation within the heated area. Spherical lenses are mounted in combination with the delivery fiber to expand and focus the laser beam to a 5-mm spot size at a working distance of 2.54±1.25 cm.

The specially designed laparoscopic handpiece was designed and built to fit within a 5-mm trocar. A laser fiber and temperature sensor cable is attached to the back of the handpiece. For compactness, the temperature sensor is incorporated into the body of the handpiece. The laser fiber and the temperature sensor fiber are co-aligned within the handpiece so that surface radiation is collected
and transmitted back to the sensor. Surgeon control of the laser beam position is accomplished by maneuvering the instrument in proximity to the target as well as by external compression and movement of the abdominal wall. An inlet port allows continuous dry carbon dioxide gas purging of the sensor and sensor fiber to prevent any moisture accumulation on the handpiece. The mesh-solder coupon is continuously exposed to the laser while the laser beam is expanded and moved slowly over the entire solder surface area to expose and completely melt the solder.

Delivery of the solder into the abdominal cavity was accomplished using a specially designed solder delivery tool. The tool is designed to fit within a 12-mm trocar. The solder-mesh construct is wound onto the shaft of the instrument before it is inserted into the trocar. A retention slot is provided to facilitate this process. The material is unwound from the tool once it has been inserted into the abdomen. The tool facilitates the precise positioning of the material at the surgical site and is sufficiently long to allow its use as a means of adjusting and maintaining the positioning of the material for initial fixation at the desired location.

**In-Vivo Model**

All animal studies were conducted in accordance with PHS guidelines and under a protocol approved by the Institutional Animal Care and Use Committee of Rochester General Hospital. A laparoscopic porcine model of intraperitoneal onlay mesh (IPOM) placement was used. Laparoscopy was accomplished using three 12-mm trocars in 24 female Yorkshire pigs weighing 20 kg to 25 kg. Anesthesia induction was carried out by using the mixture of 22 mg/kg ketamine and 1.1 mg/kg acetylpromazine intramuscularly and was maintained by Fluothane inhalation. Laparoscopy was conducted with CO₂ insufflation at 12 mm Hg. Each animal received simulated hernia closures using the study configurations according to random position assignment (Figure 1). Segments of Parietex TET, Parietex TEC, and Prolene mesh (5 x 5 cm) were embedded in 55% collagen solder. The segments were inserted using the specially designed mesh introducer instrument and fixed to the peritoneum by using the CEE laser (1.45 μ, 4.5 W CW, 5 mm spot, 55°C set temperature; or 1.533 μ, 4.8 W CW, 55°C set temperature) with the custom laparoscopic handpiece designed for this purpose. Parietex PCO mesh segments were inserted and affixed using the Endo-hernia stapler (Control). The trocar sites were closed with 0-polydioxanone sutures in the fascia, and skin incisions were closed with 0-polypropylene sutures. Each animal received ceftriaxone 50 mg/kg and Banamine 1 mg/kg IM at the conclusion of the procedure.

Figure 1. Schematic of the experimental design demonstrating the positioning of mesh segments and the locations of the trocars in the porcine model.

The animals were recovered and underwent second-look laparoscopy 6 weeks after surgery. The weld sites were assessed for integrity, adherence of the mesh, gross evidence of inflammation or tissue damage, and for the presence and severity of intraabdominal adhesions. The study sites were inspected and subjected to adhesion grading with a standardized grading scale (Table 1). The mean values ±SD were computed, and statistical analysis was conducted using both 1- and 2-tailed t tests. The mesh sites were harvested after the animals had been euthanized by intravenous pentobarbital (100 mg/kg) and po-
tassium overdose while under general anesthesia. The abdominal wall tissues were excised and fixed in 10% formalin. Representative samples were taken, embedded in paraffin and stained using Hematoxylin and Eosin (H&E), and Masson’s trichrome stain (Trichrome) staining techniques. Stained sections were evaluated by light microscopy. Histologic evaluation of the implants was conducted on representative samples from each group after paraffin embedding and thin sectioning. Standard H&E staining was performed to evaluate the tissue response, the degree of cellular inflammation and infiltrates present, as well as the overall architecture of the healing wound. Masson’s trichrome staining was performed to detect the presence of collagen deposition and to evaluate the status of the collagen solder.

RESULTS

The mesh-solder constructs were easily inserted and affixed to the peritoneum in all cases. Solder fluidity and subsequent adherence was acceptable in each of the solder fixation groups. However, the Prolene mesh tended to curl at its edges as the solder material was melted. As a result of this observation, each Prolene segment had 2 staples applied to assist in maintaining initial contact with the peritoneal surface during the soldering process.

Second-look laparoscopy and harvest was performed on the initial 4 subjects 6 weeks after implantation. All 4 subjects were healthy. The control Parietex PCO mesh was incorporated, and there were few adhesions. All of the Prolene polypropylene mesh constructs were incorporated and adherent to the peritoneal surface. All of the Prolene constructs, except for a second construct placed in subject #4, had grade IV adhesions to the liver and spleen. The extra segment had a few grade II adhesions to the omentum. The Parietex TEC, which is a flat-weave polyester mesh, exhibited similar features to those observed in the control composite material cases, with grade I-II adhesions. The Parietex TET, which is a 3D weave of polyester mesh, was dislodged in one instance and was between the stomach and liver with grade IV adhesions in both locations. It was likely that we were unable to bond enough material during the soldering process in that instance. This is most likely due to the thickness and 3D weave of this mesh material and the specific laser parameters used for solder melting in this study. The others had become densely adherent to the left lobe of the liver, the spleen, and the abdominal wall at the site of intended mesh fixation. It was hypothesized that some of the results observed in the first group of animals was due to the specific locations where the mesh constructs were placed (ie, the left upper quadrant, LUQ) rather than due to the solder or IPOM technique. No evidence of inflammation or residual collagen material was found in any of the animals at the time of second-look laparoscopy.

The type of mesh-solder constructs and the location of fixation were randomly allocated in the subsequent batches of animals to address the hypothesis that the location of implantation affected subsequent adhesion formation. As was expected, the constructs placed in the liver-spleen area formed dense adhesions regardless of the type of mesh-solder construct located in that position. This confirmed that adhesion formation in the LUQ is a function of location and motion of internal organs against the mesh that is the cause. Adhesions to the majority of the segments, including the controls, were generally grade II to omentum or none (grade 0). The location of adhesions, when present, was at the edges of the mesh, as is typically observed clinically. The Parietex PCO composite examples were more hypervascular than the solder-Parietex TEC constructs in all of the animals. Adhesions for both construct types were absent (grade 0) in the majority of the samples, with the constructs in proximity to the liver and spleen, demonstrating grade III and IV adhesions.

Histologic evaluation was completed on representative samples of control and experimental mesh constructs harvested during second-look laparoscopy 6 weeks postoperatively. Tissue histology evaluated the degree of and presence of inflammatory reaction, the presence of local tissue damage, the status of the solder, and the degree of incorporation of the mesh material. The latter parameter was evaluated by assessment of the presence of a neo-peritoneum and evidence of collagen ingrowth.

Both H&E and trichrome staining demonstrated that all of the experimental solder constructs were appropriately

| Grade | Description |
|-------|-------------|
| 0     | No adhesions present |
| I     | Filmy adhesions present at the edge of the mesh (site) |
| II    | Omental adhesions present at the edge of the mesh |
| III   | Bowel adhesions to the mesh which can be disrupted by gentle manual distraction |
| IV    | Bowel and/or other adhesions requiring sharp dissection for lysis |

Table 1. Adhesion Grading Scale
bioincorporated with no evidence of any residual solder. There was no evidence of any appreciable difference from the Parietex PCO controls. Representative histologies following H&E staining are shown in Figure 2. All of the samples exhibited a complete covering of neoperitoneum and several layers of fibroblasts with good revascularization of the material. No undue reaction occurred at the native peritoneal surface, and the mesh fibers were enveloped in a foreign body reaction with foreign body giant cells and fibrosis. The control samples appeared to be slightly more hypervascular. However, the fibrosis and foreign body reaction to the mesh material itself was the same in all instances. The tissue was oriented in a concentric layer around the mesh fibers, and the entire surface was covered with a similar linearly arranged cellular layer. These layers all stained densely for collagen. No evidence was found of damage or fibrosis of the subjacent tissue layers.

Trichrome stains showed that the collagen content was similar in each sample studied. Evidence of reperitonealization of the mesh and mesh-solder composites was readily apparent. The mesh was covered with a cellular layer containing fibroblasts that stained positively for collagen and elastic fibers. The cell types observed and the local reaction to the mesh and mesh/solder composites are consistent with the changes observed during normal healing and mimic the histology of bio-prosthetics postimplantation.

Statistical analysis of the adhesion grades and histology demonstrated that there was no difference between the Parietex PCO (controls) and the experimental mesh-solder constructs.

**DISCUSSION**

Modern hernia repair is founded on the principle of providing tissue reinforcement using prosthetic materials that permit tissue ingrowth and with fixation strategies that prevent mesh migration. Several types of open and minimally invasive techniques result in the placement of mesh materials in contact with the abdominal content to affect incisional, inguinal, or ventral herniorrhaphy. Much work has been directed at developing materials and fixation strategies that prevent the formation of adhesions to the mesh material in an effort to reduce the risk of complications secondary to postsurgical adhesion formation or due to the method of mesh fixation itself. Jenkins et al noted that the ideal prosthetic material should be chemically inert, noncarcinogenic, and capable of resisting mechanical stress, capable of being fabricated in the form required, and sterilizable, yet not be physically modified by tissue fluids, excite an inflammatory or foreign body reaction, or induce a state of hypersensitivity or allergy. The most frequently used permanent materials include meshes made of polypropylene, polyester, and ePTFE. These materials have been demonstrated to provide appropriate strength and tissue ingrowth characteristics.

The gross findings of our present study are consistent with observations regarding the normal course of wound healing in humans and animals. The collagen solder permitted...
mesh fixation to the peritoneal surface without the use of staples or suture, and without adversely affecting the rates of bioincorporation of the polypropylene and polyester mesh materials tested. The histology observed was comparable to the results observed in other studies.

The ability to cover the mesh and attach it to the peritoneal surface with a hydrophilic absorbable material is likely to facilitate intraperitoneal onlay mesh (IPOM) repair strategies and will reduce or eliminate intraabdominal adhesion formation and its attendant morbidity. Several composite materials that combine hydrophilic and permanent components are marketed for this purpose. Laser-assisted mesh fixation is a promising alternative for laparoscopic herniorrhaphy. The collagen-based solder material used in this study can be used both to affix mesh to the peritoneum and to prevent or reduce the incidence and severity of adhesions. Parietex PCO mesh is an already marketed, FDA-approved product with a PEG-treated collagen based adhesion-preventing layer (ie, gelatin). The collagen solder used in the present study is similar to the PEG-treated collagen in the currently marketed product. The proprietary collagen material can be applied to other mesh materials as we have demonstrated in the present study, or it can be used as a “stand alone” product as we have shown in earlier investigations.

The adhesion grading results in this study are similar to the results of our previous studies demonstrating that the collagen soldered constructs behave the same as the Parietex PCO composite controls behave. It is well known that monolayer or uncoated mesh causes adhesions and other potential problems like the potential for fistula formation. We demonstrated in earlier studies that there were fewer adhesions relative to uncoated meshes in the models studied. The results of our chronic porcine studies have demonstrated that there is no statistical difference between the adhesion incidence observed in solder-embedded mesh materials and the Parietex PCO composite material in this experimental model.

Van’t Riet et al. investigated the incidence of adhesions and the strength of bioincorporation of a collagen-coated polypropylene mesh (Parietien, Sofradim, France) in a murine model. These investigators created full-thickness defects in the abdominal wall and sutured the mesh intraperitoneally. They demonstrated significantly fewer adhesions and higher mean tensile strengths in the Parietien group compared with polypropylene mesh controls 30 days postoperatively. Tissue histology demonstrated acceptable tissue ingrowth and overall bioincorporation of the polypropylene material. The current study demonstrates similar results using the investigational collagen solder and polypropylene mesh.

It is noted however that the embedded Prolene mesh used in the present study tended to curl at its edges when the laser energy was applied to melt the collagen solder, under the particular parameters and constraints of this investigation. This result was due to heating of the polypropylene and its temporary deformation due to the particular weave of the native material. A minimum number of staples was used for the sole purpose of maintaining good apposition of the mesh-solder constructs, which is a necessary condition for soldering and mesh fixation to occur. We have not incurred this curling problem when using other polypropylene constructs like UltraPro in subsequent experiments (unpublished data).

Major factors that contribute to hernia recurrence following the use of prosthetic mesh techniques include insufficient mesh size or inadequate coverage of multiple hernia defects, inadequate mesh fixation, hematoma or seroma formation, folding or twisting of the material, and shear forces leading to dislodgement of the material. Collagen tissue soldering has the potential to prevent or minimize these problems by improving the ability to apply and uniformly fix the prosthetic to the abdominal wall or the peritoneal surface. The use of sutures, staples, tacks, and other permanent methods for mesh fixation will be reduced or possibly eliminated. This would reduce the incidence of complications normally associated with these fixation methods.

The ability to apply material in situ with fewer manipulations and with the use of fewer sutures or other permanent fixation devices is desirable. The solder formulations prepared for infrared laser activation in this study were composed of chemically derivatized porcine Type I collagen, bovine Type I collagen, or gelatinized collagen. Collagen was chosen due to its long history as a safe, biocompatible biomaterial, and due to its ability to be made chemically functional into a base formulation with unique cohesive and adhesive characteristics. The high concentration preparations manufactured for this study are also capable of being cast into films for subsequent sectioning into strips for application to the weld site.

This study demonstrates that laser-assisted tissue soldering is feasible and is capable of achieving intraperitoneal mesh fixation without the use of staples or permanent means of mesh fixation to tissue. Previous work from our laboratory demonstrates that temperature-controlled laser collagen soldering results in satisfactory acute tensile strengths after mesh fixation and demonstrates that adhe-
sion formation is reduced in scenarios that cover the mesh material with the collagen solder.\textsuperscript{38,39} Tensile strength measurements were conducted immediately following mesh fixation using the techniques and parameters of the current study. Acute solder bond strengths were similar in all groups (range, 261.5± 170.3 g/cm\textsuperscript{2} to 465.3± 288.2 g/cm\textsuperscript{2}) and were not statistically different from stapled controls (215.8± 117.8 g/cm\textsuperscript{2}). These values are similar to the 200 g/cm\textsuperscript{2} to 500 g/cm\textsuperscript{2} acute strengths reported for sutured or stapled peritoneal closure in experimental models.\textsuperscript{38} It is notable that C. R. Bard (PermaSorb, Davol Inc, Warwick, RI) currently markets a polylactate rivet or tack for use during laparoscopic herniorrhaphy. These tacks reabsorb over time, and studies from the literature support the fact that a permanent mesh fixator is not required to provide stable mesh fixation once bioincorporation of the mesh material has occurred.\textsuperscript{38—40}

Our current experimental model is not designed to probe for other complications of herniorrhaphy, such as internal hernia, fistula formation, and bowel obstruction, pain from foreign-body reaction around sutures and staples, or nerve entrapment syndromes. However, the absence of histological evidence of adverse tissue response or inhibition of mesh incorporation at the standard interval of 6 weeks after implantation, establishes a solid basis to conclude that the collagen solder strategy will result in superior clinical outcomes. It is reasonable to expect that long-term data on pull tests and other analyses should mirror the data for the specific mesh material used, given the fact that the solder is no longer present 6 weeks after implantation in the porcine model and when histology confirms that the mesh has been bioincorporated at that point in time. This is consistent with information that is well known and published in the literature.

This series of experiments demonstrates the feasibility of using a derivatized collagen soldering material for laparoscopic herniorrhaphy. The material and technology used in this study simplify the ability to orchestrate IPOM laparoscopic repair and may facilitate robotic applications with further development. Refinement of this promising technology and the development and improvement of delivery systems and material configurations to facilitate minimally invasive surgical approaches, including robotic applications, has great economic and social potential. Further development of this technique and these novel technologies is warranted.

\section*{CONCLUSION}

This study investigated the wound histology and degree of bioincorporation of prosthetic materials following mesh fixation to the peritoneal surface using derivatized collagen solder and a temperature-controlled near-infrared laser system in a porcine model of laparoscopic IPOM herniorrhaphy. The collagen solder permitted mesh fixation to the peritoneal surface without the use of staples or sutures, and without adversely affecting the rates of bioincorporation of the permanent mesh materials and without increasing the incidence of adhesions. The mesh-solder constructs demonstrated typical histology 6 weeks after implantation. Laser-assisted soldering is feasible for mesh fixation in laparoscopic intraperitoneal onlay mesh hernia repair. Further development of this paradigm is warranted.

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