LASER ASSISTED SOLDERING: EFFECTS OF HYDRATION ON SOLDER-TISSUE ADHESION

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ABSTRACT

Wound stabilization is critical in early wound healing. Other than superficial skin wounds, most tissue repair is exposed to a hydrated environment postoperatively. To simulate the stability of laser-soldered tissue in a wet environment, we studied the effects of hydration on laser soldered rat dermis and baboon articular cartilage. In this in vitro study, we used a solder composed of human serum albumin, sodium hyaluronate, and Indocyanine Green. A 2 μL solder droplet was deposited on each tissue specimen and then the solder was irradiated with a scanning laser beam (808 nm and 27 W/cm²). After photocoagulation, each tissue specimen was cut into two halves dividing the solder. One half was reserved as control while the other half was soaked in saline for a designated period before fixation (1 h, 1, 2, and 7 days). All tissue specimens were prepared for scanning electron microscopy (SEM). SEM examinations revealed nonuniform coagulation across the solder thickness for most of the specimens, likely a result of the temperature gradient generated by laser heating. Closer to the laser beam, the uppermost region of the solder formed a dense coagulum. The solder aggregated into small globules in the region anterior to the solder-tissue interface. All cartilage specimens soaked in saline suffered coagulum detachment from tissue surface. We noted a high concentration of the protein globules in the detached coagulum. These globules were likely responsible for solder detachment from the cartilage surface. Solder adhered better to the dermis than to cartilage. The dermal layer of the skin, composed of collagen matrix, provided a better entrapment of the solder than the smooth surface of articular cartilage. Insufficient laser heating of solder formed protein globules. Unstable solder-tissue fusion was likely a result of these globules being detached from tissue substrate when the specimen was submerged in a hydrated environment. The solder-tissue bonding was compromised as a result of this phenomenon. © 1998 Society of Photo-Optical Instrumentation Engineers. [S1083-3668(98)01104-6]

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1 INTRODUCTION

The realization of a practical endoscopic device to connect small and delicate tissues will be a major medical breakthrough and open the door to a new class of minimally invasive surgical procedures. In order to achieve this, a new generation of tissue connection techniques must be developed. New suturing techniques have been adapted for laparoscopic and endoscopic wound closure.1–3 In addition, special mechanical grasping and looping devices have been developed to simplify laparoscopic suturing.4–6 Endoscopic staples and clips have been used to control bleeding.7 These devices provide a quick tissue connection, however, they are associated with (1) fluid leakage8 and (2) inhibition of tissue growth at the repair sites, especially for young patients.9 Furthermore, these mechanical devices are difficult to maneuver for hard to reach deep lesions.10 Laser assisted tissue soldering (LATS) may provide a viable alternative to these conventional techniques for endoscopic surgery. Laser beams can be steered through endoscopic tools to virtually any target tissue, while tissue solder can be used to provide sufficient tensile support11 and deliver growth factors to speed recovery.12

One major drawback of LATS is the lack of a reliable method to assure proper solder coagulation at the tissue. In most cases, visual feedback is used to determine the status of the coagulation.13–18 Surface temperature is another feedback parameter that has been investigated.19–22 Since photocoagulation is recognized as a rate process, meaning it is governed by both temperature and the heating time during laser irradiation,23 infrared radiometry has been used to monitor solder coagulation by advancing the laser beam while advancing the laser beam when solder surface reaches a preset-control temperature.24 However, it is generally impossible to use these feedback techniques which use surface information to determine the degree of coagulation at the most crucial region of the whole process: the solder-tissue interface, because this region is buried at the bottom portion of the solder.
LATS may be used for endoscopic tissue repair where the repaired tissue is exposed to a hydrated environment inside the body. A reliable and stable solder-tissue adhesion is critical for the success of laser soldering for endoscopic surgery. The goals of this study include: (1) the investigation of albumin-based solder thermal coagulation process, (2) the study of the effects of hydration on solder-tissue interface, and (3) the comparison of substrate surface morphology on solder adhesion.

2 METHODS

2.1 SOLDER COMPOSITION

The protein solder composed of 25% human serum albumin and 0.5% sodium hyaluronate dissolved in deionized water was based on the recipe from Pohl and Bass. An absorbing dye Indocyanine Green (ICG) with a concentration of 2.5 mg/mL was added to enhance solder light absorption from a diode laser at 808 nm (Alcon Laboratory, Ft. Worth, Texas).

2.2 SOLDER DEPOSITION AND PHOTOCOAGULATION

A cotton swab was used to wipe dry the tissue surface, then a single droplet (2 μL) of tissue solder was deposited on the surface with a precision pipette. Two tissue types were used, excised rat skin dermis and baboon articular cartilage. The subdermal muscle of the rat skin specimens was carefully removed to expose the type I dermal collagen. The removal process roughened the dermal collagen matrix increasing the surface area for solder attachment. A scanning cw laser beam was achieved with a 600 μm fiber mounted onto a motorized translation stage. The scanning laser beam was used to coagulate the solder onto the tissue surface (see Figure 1). The laser parameters were 500 mW, spot diameter of 1.5 mm, and scanning speed of 0.84 mm/s. The scanning laser beam mimicked the sweeping technique commonly reported in the literature. Four passes of the laser beam were made over the solder. To test the effect of dehydration, an equal volume of solder was air dried for 3 h on a skin specimen and a cartilage specimen.

2.3 TISSUE HYDRATION AND FIXATION

Following the experiments, a full thickness incision was cut at the center of the soldered tissue exposing the solder-tissue cross section. Each specimen was cut orthogonal to the scan direction of the laser beam. One half was immediately fixed in 1% glutaraldehyde and the other was soaked in phosphate-buffered saline (PBS) for the prescribed periods before fixation. The periods were 1 h, 1, 2, and 7 days. One specimen was used for each hydration period and tissue type.

2.4 SEM ANALYSIS

Preparation for SEM analysis included specimen dehydration in graded ethanols, critical point drying, and specimen coating with a thin layer of gold-palladium. SEM analysis was performed on a Philips 515 scanning electron microscope.

3 RESULTS

3.1 CARTILAGE SPECIMENS

The solder was attached to the cartilage surface for four out of five control specimens that were fixed immediately after coagulation. Discoloration or "desiccation" was noted among most of the solder coagulum. Two regions of coagulated solder were identified (see cross section of solder in Figure 2, left panel). The right panel of Figure 2 shows the boundary between these regions. The region of the solder closer to the tissue substrate was mainly composed of protein globules. The diameter of these globules was in the range of 2–3 μm. The band of solder closer to the laser beam was more packed and fully coagulated. This band seemed to be composed of protein globules that were densely fused together.

After soaking in PBS for 1 h, the center region of the solder detached from the cartilage surface (Figure 3, left panel). Some areas of the cartilage from which the bulk of solder had detached still carried a remnant of the protein globules. However, the region of the cartilage at the center part of the solder was almost free of solder globules. The size and shape of protein globules among the soaked specimens and the control specimen were approximately the same. Solder detachment was also noted for the specimens that had been hydrated for one and two days. After soaking for one week, one side of the solder was almost fully detached from the cartilage surface (Figure 4). Moreover, there was shrinkage from within this coagulum since "wrinkles" could be seen along the outside of the solder. There was no evidence of globule remnant on the cartilage once the solder was detached. Looking from the posterior side, the detached solder seemed to be un-
organized with frayed solder globules suggesting that some of the protein globules might have been washed away.

For the dehydrated specimen, the solder detached from the tissue substrate during the glutaraldehyde fixation process. The solder seemed to be very densely packed at the cross section. The air-dried solder showed no evidence of any globule formation at the area where the solder was attached to the cartilage surface (Figure 5).

3.2 SKIN SPECIMENS

The solder appeared to be attached to the dermis for all five control specimens that were fixed right after laser irradiation. Similar to the cartilage specimens, two regions of denatured solder were identified (Figure 6, left panel). There was a densely coagulated band near the anterior part of the solder closer to the laser irradiation. This coagulated band was formed by the coagulation of individual globules. Beyond the coagulated band, solder globules were found at the solder/dermis interface. These protein globules had diameters of about 2–3 μm (Figure 6, right panel).

The solder-tissue interface had several small gaps at the globular region of the solder soaked in PBS for 1 h. The specimen that was soaked for one day had a larger opening at the solder than the 1 h
Some steam vacuoles appeared at the upper solder region as a result of boiling of the solder during the photocoagulation process. The protein coagulum that defines the outlines of the steam vacuoles was densely coagulated with no globule formation (Figure 7, right panel). Solder detached from the specimen soaked for two days. Similarly, the specimen that was soaked for seven days had a few openings at the solder-tissue interface (Figure 8). Again, these openings occurred at the globular region where the solder had separated from itself. Similar to the dehydrated cartilage specimen, no sign of globule formation was found in the dehydrated solder on the skin specimen.

**Fig. 6** Rat skin specimen—control. Left panel was taken at 356 x with Bar scale of 50 μm per increment. Note that nonuniform coagulation occurred across the cross section of the solder-tissue interface. The magnification of the right panel is 2955 x. Similar to the cartilage specimens, globules and fusion of these globules were noted.

**Fig. 7** Rat skin specimen—after one day of hydration. Left panel shows steam vacuoles produced at the solder coagulum. Some globules were located at the base of the solder coagulum that may have created an unstable bond between the solder and tissue interface. Left panel is 89 x and right panel is 734 x. Bar scale at the left panel is equivalent to 500 μm.

**Fig. 8** Rat skin specimen—after seven days of hydration. Original magnification: left panel is 178 x and right panel 890 x. Bar scale is 50 μm at the left panel. Despite protein globules appearing at the solder-dermis interface, the solder coagulum was still attached to the majority of the dermal substrate surface.

### 4 Discussions

The laser parameters and ICG concentration used in this study were typical of values reported in the literature that produce reasonable solder welds of tissue. However, the SEM results of this study suggest that the solder was not completely coagulated at the solder-tissue interface. Nevertheless, we did obtain consistent and stable control welds. Furthermore, some of the hydrated welds on rat dermis were stable. For both tissue types, we observed protein globules at or near the solder-tissue interface. For the smooth articular cartilage surface, these globules were associated with solder detachment. In contrast, detachment did not always occur at solder-dermis interfaces. The process of scraping away the subdermal muscle layer to expose the rat skin dermal collagen roughened the dermal collagen matrix and may have entrapped the solder globules. Therefore, a roughened substrate is more desirable surface for tissue soldering.

If insufficient heating at the solder-tissue interface causes weak globule welds, is more energy deposition a solution to the problem? Although solder was coagulated into a dense coagulum at the upper portion of the solder coagulum and sufficient energy was deposited to cause steam vacuoles in the coagulated solder (see Figure 7), solder detachment was still observed beneath the well coagulated upper portion. The absorption of laser light by a solder droplet created a temperature gradient transversely across the solder. A dense coagulum was formed in the “hotter” anterior portion of the solder. Insufficient coagulation in the posterior portion prevented globules from developing into a dense coagulum. The dense coagulum and the steam vacuoles at the upper solder portion may have formed a thermal barrier, reducing heat conduction to the lower globular portion. As a result,
the most critical part of the weld, the solder-tissue interface region, experienced insufficient coagulation. This unstable region was the site of solder detachment over time after hydration.

Discoloration or desiccation of the solder coagulum was noted during photocoagulation. This was not sufficient to ensure fusion of protein globules at the solder-tissue interface. Recently, infrared thermal feedback was used to control the welding process. Both the visual and thermal feedback techniques monitor solder surface information over time as a means to determine coagulation end point. Surface information although necessary is not sufficient to determine the degree of coagulation achieved at the solder-tissue interface. If the temperature gradient with depth is small, then surface information can provide a reasonable estimate of the coagulation at the critical solder-tissue interface. The temperature gradient can be reduced by:

1. using a smaller solder droplet volume to obtain a thinner solder;
2. reducing absorber concentration and adjusting laser parameters; or
3. applying temperature control to avoid boiling of solder which causes steam vacuoles that impair thermal conduction.

5 CONCLUSIONS

We have studied the coagulation process of albumin tissue solder. Upon heating, albumin solder first aggregates into globules, then these globules fuse together. With more energy applied, the fused globules become a more densely packed coagulum. However, steam vacuoles may arise from moisture vaporization inside the solder.

Inadequate heating of solder induced globule formation. Globules initially attached to the tissue substrate detached once the specimen was submerged in a hydrated environment. This phenomenon either significantly weakened or completely destroyed the bonding of the solder to tissue.

We believe that the key to a stable weld is a dense coagulum at the solder-tissue interface. Unless a low solder temperature gradient is ensured, surface feedback parameters will not be sufficient to control the degree of coagulation at the critical solder-tissue interface.

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