Mechanical Properties of Coagulated Albumin and Failure Mechanisms of Liver Repaired with the Use of an Argon-Beam Coagulator with Albumin

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Abstract: Hemostasis in the traumatized liver has been achieved by thermally denaturing topically applied albumin. In this article, the mechanical properties of liver and denatured albumin (solder) were measured, and the failure methods of liver repaired with albumin were identified. The ultimate tensile strength and Young’s modulus were measured for healthy liver (N = 20) and thermally damaged liver (N = 20). The ultimate tensile strength and Young’s modulus were measured for three concentrations of coagulated albumin (25, 38, and 53%) in a single layer and for two layers of denatured 38% albumin. Failure under tension of argon-beam coagulator soldered liver on the parenchymal surface (N = 30) with 38% albumin in two layers had a 70% occurrence for tearing at a mean stress of 39 kPa and a 23% occurrence for shearing at a mean stress of 7 kPa. Liver repaired on the interior surface (N = 11) failed in tension by tearing (64%) at a mean stress of 34 kPa and by shearing (36%) at a mean stress of 6 kPa. Argon-beam coagulator soldering with 38% albumin took 6 s/cm² for two layers of solder and gave the best balance of usability, strength, and matching of mechanical properties with those of the liver.

Keywords: argon-enhanced electrocautery (AEC); tissue welding; solder; liver trauma

INTRODUCTION

The liver is the second most commonly injured organ in both penetrating and blunt injuries. Controlling hemorrhage of injured liver is a problem that “is primarily a mechanical one of closing holes in large intrahepatic blood vessels and controlling oozing from innumerable fine bleeding points on the damaged liver surface as expeditiously as possible.” Current treatment methods offer moderate success at controlling hemorrhage. Most commonly, severe liver injury treatment involves compressive gauze packing, where gauze surrounds the liver filling the abdomen. Other options include suturing of bleeding points, placing mattress sutures, ligating hepatic arteries, or resecting segments or lobes of liver. A more recent approach to achieve hemostasis after liver injury is to use an argon-ion beam coagulator (herein referred to as ABC).

The ABC is an argon-enhanced electrosurgery unit. The ABC uses a noncontact monopolar coagulator, which conducts radio-frequency current to the tissue along a jet of inert and non-flammable argon gas. Argon has a lower breakdown voltage than air; therefore the current ionizes the gas within the argon jet (making it blue in color). It is the arcing of current into tissue that causes coagulation. The gas directs the flow of current. Furthermore, the blowing gas has been credited with allowing better visualization by clearing blood from the tissue and reducing smoke by limiting the availability of oxygen at the desiccation site. Application of the ABC to the liver alone does not bond adjoining tissues together; however, ABC complimented with albumin solder does bond adjoining tissues. In this article, the materials associated with ABC repair with albumin are evaluated. The mechanical properties of coagulated albumin for 25, 38, and 53% concentrations are measured to failure. The mechanical properties of native and thermally damaged liver are measured and compared to coagulated albumin. Finally, the strength of liver repaired with ABC and two layers of 38% albumin are measured and the failure mechanisms are established.

MATERIALS AND METHODS

The materials and methods are organized as follows: First, a description of the argon beam coagulator and settings used...
throughout the experiments are given. Next, the albumin concentration protocol is detailed, followed by the details of albumin coagulation into dog-bone samples with the use of the argon-beam coagulator. Liver preparation for native and thermally damaged samples follows. Next, the procedure for making repaired liver samples with albumin solder is given. Finally, methods for mechanical testing of the various materials are presented.

**Argon-Beam Coagulator**

The argon-beam coagulator (ABC) (Force 2 Electrosurgery and Force GSU System, Valleylab, Boulder, CO) consisted of an electrosurgery unit, gas unit, hand piece, and a grounding pad. The standard grounding pad (Valleylab), a sticky disposable metallic pad approximately 12 × 20 cm in dimension, was stuck onto an aluminum plate (20 × 30 cm) that could be washed and reused. The flow rate for the argon gas was set to 4 l/min to minimize the risk of embolism, because the flow rate was found to have no measurable effect on the quality of the repair or on ease of ignition of the ion beam. The ABC unit was used with a coagulation setting characterized by a low duty cycle (≈ 6% on and 94% off) of 390-kHz damped sinusoids burst at 30 kHz at 8500 V peak to peak. Power settings above 60 W caused the liver to appreciably shrink in vitro, and so all experiments were performed at 60 W. Regardless of the power setting the energy delivered remained at 350 ± 36 J/cm², with color change of the albumin used as an endpoint.

**Sample Preparation**

**Albumin Concentration.** Albumin was prepared primarily into 38% and 53% w/v (100% = 100 g/100 ml) from standard sterile 25% human serum albumin. Excess water was removed from the albumin with the use of pressure filtration in a sealed Amicon chamber with a 25-kDa filter that allows the passage of water molecules but not the larger protein molecules. The filter was presoaked for 5 min in deionized/distilled water. 200 ml of 25% albumin was poured into the assembled Amicon chamber, stirred by a magnetic stirring bar, and then sealed with the chamber top. The top of the chamber was connected to a Millipore filtered nitrogen gas line at 60–65 psi. The temperature of the chamber was maintained at 57 °C to reduce viscosity and facilitate stirring. The albumin was slowly stirred to prevent the surface from becoming solid. Filtration took 12 and 48 h for 38% and 53% albumin, respectively.

The concentration of the albumin (w/v) at 24 °C was determined with the use of a refractive index measurement with an Abbe refractometer. A calibration experiment comparing index of refraction to concentration led to the relation (valid at 24 °C.)

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\% \text{ concentration} = 575(n_{\text{albumin}} - n_{\text{water}}),
\]

where \( n_{\text{albumin}} \) is the measured refractive index of the albumin solution and \( n_{\text{water}} = 1.333 \) is the refractive index of water at 24 °C. When the desired concentration was achieved, the albumin was drawn into sterile 6- or 10-cc syringes, capped, and stored in a refrigerator.

**Albumin Coagulation.** To choose the concentrations of interest in this study, multiple concentrations of albumin ranging from 34 to 52% in 2% increments were prepared and compared qualitatively. The solder was assessed according to the strength of its bond with the liver after ABC application. The solder was placed onto a vertical surface of raw liver, allowed to spread for 5 s due to gravity, then heated with the ABC until the albumin turned golden brown in color. If the denatured albumin could be easily pulled away from the liver surface after this color change, this indicated that the albumin solder layer was too thick to denature completely. The albumin–liver interface never coagulated, and thus no bond was formed. This lack of bonding was common for concentrations above 40%; however, bonding could be achieved when the albumin was manually spread to create a thinner layer of solder. And so, albumin was quantitatively compared for its ultimate tensile strength and Young’s modulus at concentrations of 25% (commercially available concentration), 38% (below the limit requiring manual spreading), and 53% (below the limit where albumin becomes a solid at room temperature).

Solid albumin samples were formed by denaturing the albumin solder with the ABC in a Teflon mold until golden brown in color. The mold was made from 1-mm-thick Teflon sheets that had a dog-bone shaped hole (4 cm long, 4.5 and 9 mm wide at the middle and end sections, respectively). This hole was backed by two layers of masking tape. The mold was filled with albumin and placed onto the grounding pad of the ABC. The masking tape allowed the conduction of current through the albumin, causing coagulation. A single layer of masking tape caused charring of the albumin due to violent arcing of current; three layers of tape produced a weak ion beam. The denatured albumin dog bone was extracted from the mold by removing the tape and pressing the albumin from the Teflon with an aluminum die (the same die used to cut the mold in the Teflon).

**Liver.** All liver in this study was porcine liver obtained fresh from a supermarket on the day of the slaughterhouse shipment. The liver was filleted into large area sheets less than 5 mm thick, with the parenchymal surface left intact. The liver sheet was then cut into several small dog-bone pieces approximately 4 cm long and 2–4 mm wide in the middle and 1 cm or more wide at the ends. The liver sections were placed onto a tray with paper towels saturated with phosphate-buffered saline (PBS) and covered with paper towels and PBS until tested for tensile failure.

Twenty randomly selected samples from the same liver as the native samples were immersed in a 98 °C water bath for 2 min to cause thermal damage. The cross-sectional area of
the liver did not significantly change after soaking in a hot water bath ($p = 0.5$).

**Soldered Liver.** Liver was repaired with the use of 38% albumin in the following process. Four fillets of liver, approximately 4–5 cm wide by 10 cm long by 4 mm thick were cut in half down the length making two long strips from each fillet 10 cm long and 4 mm thick. The two halves were realigned and then albumin was applied from the syringe in a single line down the incision. The 38% albumin naturally spread in a 1.5-cm wide strip centered on the incision. The ion beam was applied down the length of the incision and along each side of the incision. The albumin turned white almost instantaneously. Ion-beam application continued until the surface of denatured albumin turned a golden brown color with spots of dark brown charring. A second layer of albumin was applied over the first and coagulated with the ABC with the use of the same color change end point. Shorter applications of the ABC did not produce suitable repairs. Typically, these repairs failed at the bond between the albumin and the liver. Moreover, close examination showed that the albumin did not denature completely down to the liver surface.

The repaired liver fillets were cut perpendicular to the repaired incision into approximately ten strips (1 × 5 and 4 mm thick) from each fillet. A total of 30 samples of liver were soldered on the parenchymal surface, and another 11 samples were soldered on the interior surface of the liver. The repaired strips were stored on a tray with paper towels saturated with PBS (phosphate-buffered saline) and covered with additional PBS-soaked paper towels until tested.

To assess the quality of the repair, several details were tracked: duration of ABC use, area of the solder, the ultimate tensile strength of the repair, the depth of necrosis, and the method of failure. Depth of necrosis based on blanching of the liver was measured after tensile testing of each sample.

**Sample Testing**

Coagulated albumin was tested under tension to failure on a materials tester (No. 395.20A-02, MTS Tytron, Eden Prairie, MN). The albumin was gripped with the use of screw-down clamps. The samples were pulled 1.5 cm to ensure failure at a rate of 3 mm/s. The healthy and thermally damaged livers were loaded in tension to failure with the use of a materials tester (V1000 Chatillon, Hampshire, UK). The samples were held in the tester with the use of clamps made from medium-size binder clips (No. 72050, ACCO USA, Inc., Wheeling, IL). The upper clip was connected to a 5000-g load cell (SM-10, Interface, Inc., Scottsdale, AZ). Three samples failed near the region clamped by the binder clips; these were not included in this study. All samples were pulled a distance of 2 cm to ensure failure at a rate of 2 mm/s.

The repaired liver strips were glued (Prism 4081, Loctite Corp., Rocky Hill, CT) to aluminum plates with a 3-mm gap between plates centered on the incision (Figure 1) for mechanical testing. The glue was allowed to set for 30 s before the sample was placed in the mechanical tester. The aluminum plates were grasped by screw-down clamps. The load cell, pulling distance, and rate were the same as used for the healthy and thermally damaged liver samples.

**RESULTS**

A histogram of the ultimate tensile strength of the healthy and thermally damaged liver is shown in Figure 2. Liver that was
thermally damaged failed at a significantly lower ($p = 0.002$) ultimate strength from healthy liver in a paired $t$ test. Typical stress-strain curves are shown for healthy and thermally damaged liver in Figure 3. The stress-strain curve of healthy liver is approximated by two linearly elastic regions with a lower Young’s modulus of $360 \pm 230$ kPa and a higher Young’s modulus of $1700 \pm 870$ kPa. The thermally damaged liver was approximated as a linearly elastic material with a mean Young’s modulus of $390 \pm 200$ kPa. Also shown was a typical stress-strain curve for two layers of denatured 38% albumin loaded in tension at a speed of 3 mm/s until failure. The albumin had a mean Young’s modulus of $3900 \pm 1900$ kPa at 3% strain (strain = $\Delta L/L$, where $L$ is the length of the material).

Histograms of the ultimate strength for a single layer of denatured albumin in a single layer of 25, 38, and 53% (top to bottom) albumin solder had mean ultimate strengths of $82 \pm 72$ kPa, $180 \pm 80$ kPa, and $420 \pm 150$ kPa, respectively. For each concentration of albumin, 20 samples were tested, except for 53% albumin which had 21 samples.

Histograms of the ultimate strength for a single layer of denatured albumin are shown in Figure 4 for 25, 38, and 53% albumin. Figure 5 shows the distribution of ultimate strength for albumin denatured in two layers. A $t$ test confirmed that the mean ultimate strength for the single layer and double layer denatured 38% albumin were not significantly different ($p = 0.3$). A typical stress-strain curve for two layers of 38% denatured albumin is presented in Figure 3. The two layers of coagulated albumin had a mean Young’s modulus of 3900 ± 1900 kPa (strain = 0.03) in 19 samples and failed at a mean strain of 0.12 ± 0.07. The lowest strain at failure was 0.037, and the lowest stress at failure was 57 kPa.

Qualitative testing of the albumin concentration was performed after several soldering experiments on liver, which

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**Figure 3.** A typical stress-strain curve for tensile loading of healthy and thermally damaged liver at a load speed of 2 mm/s until failure. The stress-strain curve of healthy liver could be approximated by two linearly elastic material regions, one with a lower Young’s modulus of $360 \pm 230$ kPa and a higher Young’s modulus of $1700 \pm 870$ kPa. The thermally damaged liver was approximated as a linearly elastic material with a mean Young’s modulus of $390 \pm 200$ kPa. Also shown was a typical stress-strain curve for two layers of denatured 38% albumin loaded in tension at a speed of 3 mm/s until failure. The albumin had a mean Young’s modulus of $3900 \pm 1900$ kPa at 3% strain (strain = $\Delta L/L$, where $L$ is the length of the material).

**Figure 4.** Histograms for the ultimate strength of denatured albumin in a single layer of 25, 38, and 53% (top to bottom) albumin solder had mean ultimate strengths of $82 \pm 72$ kPa, $180 \pm 80$ kPa, and $420 \pm 150$ kPa, respectively. For each concentration of albumin, 20 samples were tested, except for 53% albumin which had 21 samples.

**Figure 5.** A typical stress-strain curve for two layers of denatured 38% albumin loaded in tension at a speed of 3 mm/s until failure. The albumin had a mean Young’s modulus of $3900 \pm 1900$ kPa at 3% strain (strain = $\Delta L/L$, where $L$ is the length of the material).

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**TABLE I.** A Summary of Ultimate Strength and Young’s Modulus Measurements. The Last Column Indicates the Strain at Which Young’s Modulus was Measured. The Albumin Results are for a Single Layer Except as Indicated.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Type</th>
<th>Ultimate Strength [kPa]</th>
<th>Young’s Modulus [kPa]</th>
<th>Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>Native</td>
<td>280 ± 150</td>
<td>360 ± 230</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1700 ± 870</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>Cooked</td>
<td>160 ± 70</td>
<td>390 ± 200</td>
<td>0.10</td>
</tr>
<tr>
<td>Albumin</td>
<td>25%</td>
<td>82 ± 72</td>
<td>1200 ± 770</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>38%</td>
<td>180 ± 80</td>
<td>1200 ± 770</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>38% (2 layers)</td>
<td>210 ± 100</td>
<td>3900 ± 1900</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>53%</td>
<td>420 ± 150</td>
<td>5100 ± 1300</td>
<td>0.03</td>
</tr>
<tr>
<td>Repair</td>
<td>Tear Failure</td>
<td>37 ± 12</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Bond Failure</td>
<td>7 ± 4</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
indicated that higher concentrations of albumin provided stronger repairs. At 40% and below, the albumin flowed into a uniform sheet, and when denatured the albumin could not be pulled away from the liver without tearing the liver surface. It was also noted that the depth of thermal damage to the liver as indicated by a color change (whitening) did not vary significantly from 1 mm, regardless of albumin concentration. This was consistent with the uniform energy deposition per area of $350 \pm 36 \text{ J/cm}^2$ required to turn the albumin golden brown.

Liver repaired on the parenchymal (exterior) surface using the ABC and 38% albumin in two layers is presented in Figure 6 and on a nonparenchymal surface (interior) in Figure 7. On the parenchyma, repairs failed 70% of the time by tearing the albumin along the incision and in 23% the albumin bond to the liver failed (Figure 8). The remaining 7% (two samples) failed at the superglue holding the liver to the aluminum plates and partially tore the albumin, so the samples were not included in either group. The mean tensile stress for tearing was $39 \pm 13 \text{ kPa}$ and $7.4 \pm 3.8 \text{ kPa}$ when the albumin bond to the liver failed. The failure method was similar on the interior surface of the liver where the tearing of the albumin had a 64% failure rate and the bond between the liver and the albumin a 36% failure rate. The mean tensile stress for tearing was $34 \pm 8 \text{ kPa}$ and $5.9 \pm 4.7 \text{ kPa}$ when the albumin bond to the liver failed for repairs on the interior surface. Liver that was repaired with the ABC, but without the albumin, did not bond together.
DISCUSSION

Tensile loading of liver repaired with ABC and albumin solder failed by one of two methods, regardless of which surface of the liver was repaired. Approximately two-thirds of repairs failed when the albumin tore along the laceration. The remainder of failures occurred when the albumin pulled free from the liver surface. The latter failures did not exhibit evidence of liver remaining attached to the albumin; however, microscopy was not performed to confirm this result. Stresses differ between the failure types due to the cross-sectional area.

ABC soldering took a mean repair time of 5.8 ± 0.6 s/cm² at a power setting of 60 W to denature two layers of albumin corresponding to a mean total energy of 350 ± 36 J/cm² delivered. ABC power was limited to 60 W in vitro because the tissue samples were significantly smaller than the ABC grounding pad. In vivo, powers may be higher than 60 W, thereby decreasing the time it takes to coagulate the albumin. Even so, ABC repair time was roughly twice as fast as the 12 ± 5 s/cm² for the laser weds achieved by Wadia et al.

ABC use has been reported to cause fatal and near-fatal venous emboli. ECRI documented three cases of gas embolism that occurred during laparoscopic procedures and concluded with recommendations for ABC use that minimize the risk of emboli. One consistent trend in this problem is a high gas flow rate. Palmer et al. show gas emboli increasing with increasing flow rate. The gas is often used to clear blood from the field. Albumin used in conjunction with ABC could potentially minimize the risk of emboli; surgeons would need to clear the surgical field (with gauze) before applying the albumin, eliminating the associated risk of using the argon gas to clear blood from the field.

The qualitative assessment of albumin concentration showed that 38% was the easiest to apply. Poppas et al. reported that concentrations less than 45% allowed easy handling and distribution to tissue edges. Though stronger, 53% albumin required manual spreading to achieve a stronger bond than 38% albumin. Use of 38% albumin eliminated operator error in deciding what constituted a sufficiently thin layer for an adequate repair; use of 38% albumin also avoided albumin sticking to the surgeon’s gloves.

Coagulated 38% albumin also roughly matches the mechanical properties compared to native liver. The Young’s modulus of albumin was similar (about a factor of 2) to that of healthy liver when strained more than 10%. Likewise, the mean ultimate strength of 38% denatured albumin (in two layers) was comparable to healthy liver failing at 210 ± 100 kPa and 280 ± 150 kPa, respectively. Higher concentrations of albumin are stiffer than native liver, and lower concentrations of albumin had lower ultimate tensile strength than native liver. Repaired liver with 38% albumin solder failed by tearing with a mean ultimate strength of 39 ± 13 kPa, much lower than either the liver or albumin alone. One distinction

Figure 8. Repaired liver with albumin solder failed by one of two methods: The albumin tore along the incision (upper) or the albumin detached from the liver surface (lower).
between liver and albumin was the strain at failure, where liver failed with a mean strain of 0.27 ± 0.06 and albumin at 0.12 ± 0.07 strain (p = 0.001).

The ultimate tensile strengths of coagulated 38% albumin in a single and double layers were not significantly different (p = 0.3). Two layers of albumin required more force to fail, because the cross-sectional area of the solder was greater and did not increase thermal damage in the liver. ABC soldering with 38% albumin took 6 s/cm² to coagulate two layers of solder and gave the best balance among ease of use, strength, and matching of mechanical properties with those of the liver.

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REFERENCES