Mechanical properties of repaired liver using an argon beam coagulator with albumin

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ABSTRACT

A new method for tissue soldering using an argon ion beam coagulator (ABC) and human serum albumin is presented. The ABC is widely used in surgery and provides a fast and precise means of achieving hemostasis. In this paper, 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 was measured for healthy liver (N=37) and thermally damaged liver (N=32). The ultimate tensile strength was 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/cm^2 for two layers of solder and gave the best balance of usability and strength.

Keywords: Argon enhanced electrocautery (AEC), tissue welding, solder, liver trauma

1. INTRODUCTION

The liver is the second most commonly injured organ in both penetrating and blunt injuries.[1] 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".[2] Current treatment methods offer moderate success at controlling hemorrhage.[3] Most commonly, severe liver injury treatment involves compressive gauze packing, where gauze surrounds the liver filling the abdomen.[3] Other options include suturing of bleeding points, placing mattress sutures, ligating hepatic arteries, or resecting segments or lobes of liver.[3] A more recent approach to achieve hemostasis after liver injury is use of an argon ion beam coagulator (herein referred to as ABC).[4]

The ABC is an argon-enhanced electrosurgery unit. The ABC uses a non-contact monopolar coagulator that 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 arcs through the gas beam, ionizing the argon (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 attributed for better visualization by clearing blood from the tissue[5] and for a reduction in smoke by limiting the availability of oxygen at the desiccation site.[6]

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 paper, 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.

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2. MATERIALS AND METHODS

2.1. 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 by 20 cm in dimension, was stuck onto an aluminum plate (20 by 30 cm) that could be washed and reused. The flow rate for the argon gas was set to 4 liter/minute to minimize the risk of embolism,[7] since 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 the coagulation setting characterized by a low duty cycle ($\approx 6\%$ 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 $350 \pm 36\,\mathrm{J/cm^2}$ using color change of the albumin as an endpoint.

2.2. Sample Preparation

2.2.1. Albumin

Albumin was concentrated to 38% and 53% w/v ($100\% = \frac{100\,\mathrm{g}}{100\,\mathrm{ml}}$) from standard sterile 25% human serum albumin. Excess water was removed using a sealed Amicon chamber with a 25 kDa filter. The filter was presoaked for 5 minutes in deionized/distilled water. 200 mL of 25% albumin was poured into the assembled Amicon chamber, followed 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. The albumin was slowly stirred to prevent the surface from becoming solid. Filtration took 12 and 48 hours for 38% and 53% albumin respectively.

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

$$\%\,\mathrm{concentration} = 575(\mathrm{n_{albumin}} - \mathrm{n_{water}})$$

where $\mathrm{n_{albumin}}$ was the measured refractive index of the albumin solution and $\mathrm{n_{water}} = 1.333$ 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.

Solid albumin samples were formed by denaturing albumin in a Teflon mold until golden brown in color. The 1 mm thick Teflon mold had a dogbone shape (4 cm long, 4.5 and 9 mm wide in the mid-section and ends respectively) and was backed by two layers of masking tape and placed onto the grounding pad of the ABC. A single layer of masking tape caused charring of the albumin due to violent arcing whereas three layers produced a weak ion beam. The denatured albumin dogbone 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).

2.2.2. Liver

All liver in this study was porcine liver obtained fresh from a supermarket on the day of the slaughter house shipment. The liver was filleted into large area sheets less than 5 mm thick keeping the parenchymal surface. The liver sheet was then cut into several small dogbone pieces approximately 4 cm long and 3–4 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.

Thermally damaged liver was immersed in an 80° C water bath for 2 minutes for 16 samples and for 12 minutes with another 16 samples.

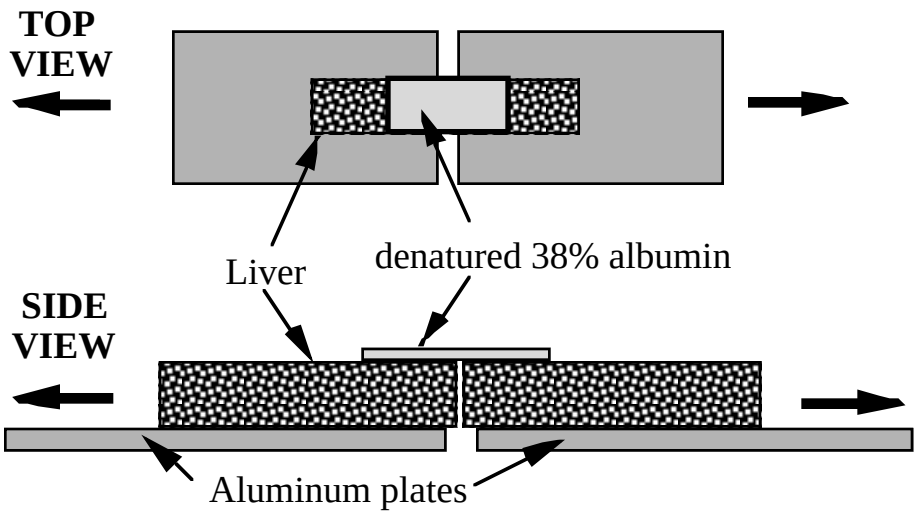

Figure 1. Soldered liver samples were mounted to aluminum plates and loaded in tension in the direction of the large arrows until failure.

2.2.3. Soldered Liver

Concentrations of albumin ranging from 34-52% in 2% increments were prepared and compared qualitatively. Solder was assessed according to how it bonded to the liver after ABC application. The solder was placed onto a vertical surface of raw liver then heated with the ABC until the albumin turned a golden brown color. If the denatured albumin could be easily pulled away from the liver surface after this color change, then it was too thick to denature completely and form a solid bond to the liver surface. This was common for concentrations above 40% and bonding was only achieved when the albumin was manually spread to create a thinner layer.

Liver was repaired using 38% albumin in the following process. Four fillets of liver, approximately 4–5 cm wide by 10 cm long by 4 mm thick was cut in half down the length making two long strips 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. The ion beam was applied to the area of denatured (white) albumin until the surface 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 using the same color change endpoint. Shorter application of the ABC did not produce a suitable repair, leading to a greater proportion of failures where the albumin failed to bond to the liver. For this case, the albumin did not denature completely down to the liver parenchyma.

The repaired liver fillets were cut perpendicular to the length of the fillet into approximately ten strips (1 cm by 5 cm and 4 mm thick) from each fillet. A total of 30 samples of liver were soldered on the parenchymal surface while 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 and covered with paper towels and PBS 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 was measured after tensile testing the repair based on color change in the liver.

2.3. Sample testing

Coagulated albumin was tested under tension to failure on a materials tester (# 395.20A-02, MTS Tytron, Eden Prairie, MN). The albumin was gripped using 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 liver were loaded in tension to failure using a materials tester (V1000 Chatillon, Hampshire, UK). The samples were held in the tester using clamps made from medium-size binder

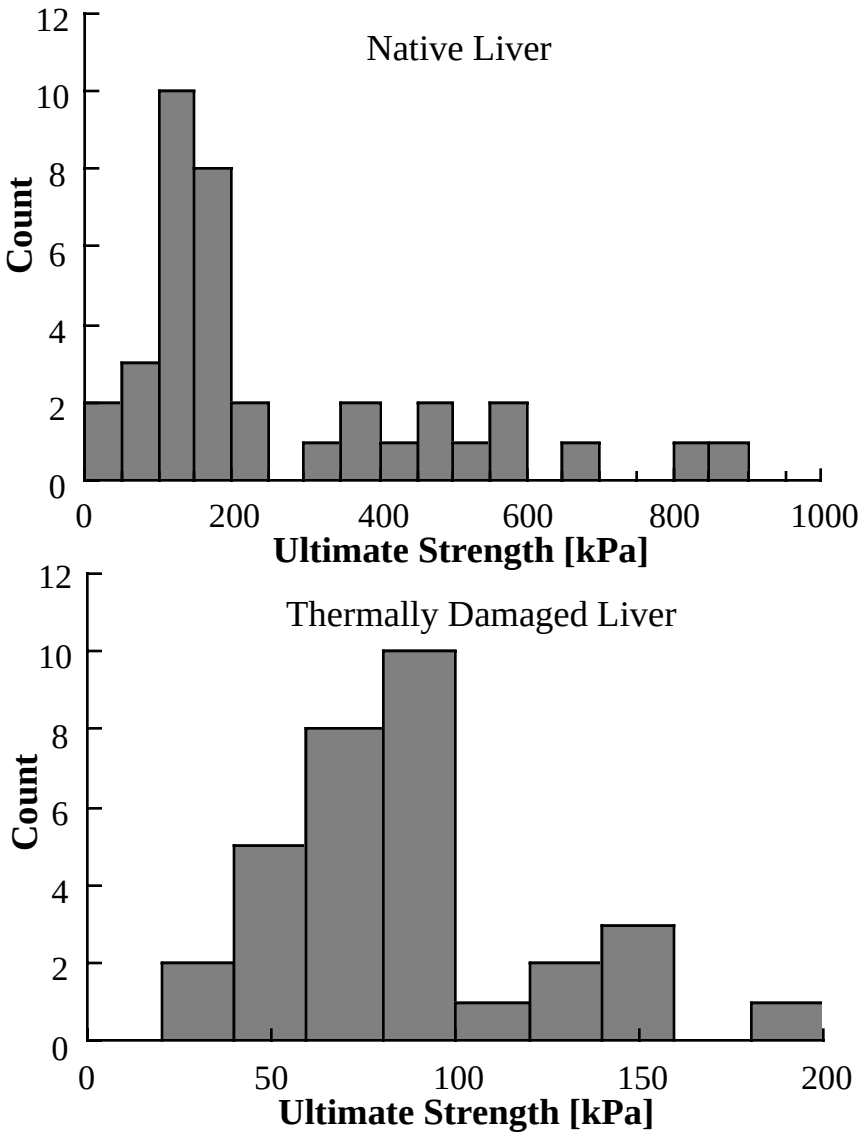

Figure 2. The distribution of ultimate strengths of liver without (N=37) and with (N=32) heating. The mean cross-sectional area of unheated liver was $20 \pm 15\,\mathrm{mm}^2$. The ultimate strength for unheated liver had a mean of $270 \pm 220\,\mathrm{kPa}$; thermally damaged liver was $88 \pm 37\,\mathrm{kPa}$ with a mean cross-sectional area of $48 \pm 18\,\mathrm{mm}^2$.

clip clips. The upper clip was connected to a 500 g load cell (Transducer Techniques, Temecula, CA). Some samples failed near the region clamped by the binder clips; these were not included in this study. The samples were pulled at least 2 cm to ensure failure at a rate of 2 mm/s.

The repaired liver strips were super-glued to aluminum plates with a 3 mm gap between plates centered on the incision (fig. 1). Screw down clamps gripped the aluminum plates in alignment. The load cell, pulling distance, and rate were the same as used for the healthy liver samples.

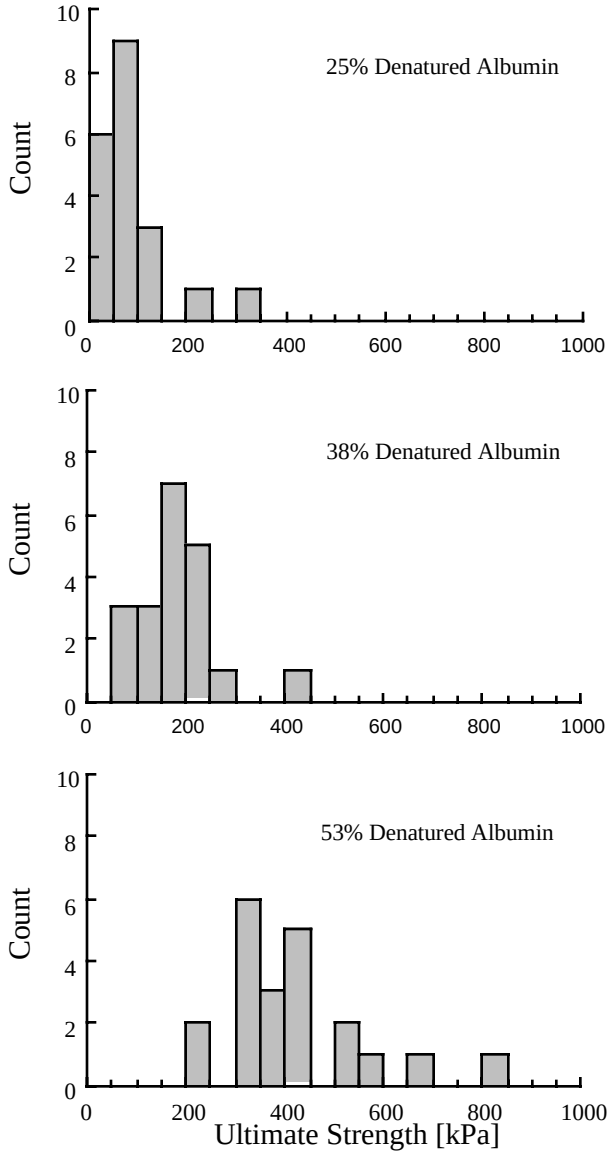

Figure 3. Histograms for the ultimate strength of denatured albumin in a single layer of 25, 38 and 53% (top to bottom) albumin solder had mean ultimate strengths of 82 ±72 kPa, 180 ±80 kPa, and 420 ±150 kPa, respectively. For each concentration of albumin, 20 samples were tested except for 53% albumin which had 21 samples.

3. RESULTS

A histogram of the ultimate tensile strength of the healthy liver is shown in figure 2. The large variation of the ultimate strength may have been due in part to differences in freshness of different livers. The age of the liver affected the *in vitro* mechanical properties dramatically. One liver that was brown with age never had a ultimate tensile strength above 38 kPa and was not included in this study. Liver that was thermally damaged by cooking for 2 minutes was not significantly different ($p = 0.2$) from liver that was cooked for 12 minutes. A histogram of the ultimate strength for thermally damaged liver is shown in figure 2.

The ultimate strength histograms for a single layer of denatured albumin are shown in figure 3 for 25, 38, and 53% albumin. Figure 4 shows the distribution of ultimate strength for albumin denatured in two layers. A

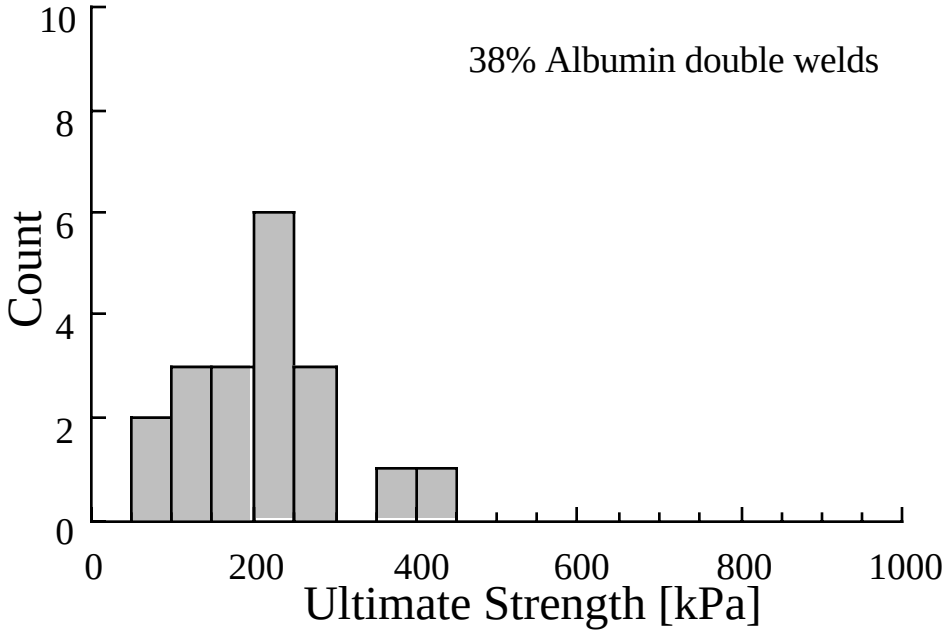

Figure 4. The 19 samples of two layers of denatured albumin solder did not have a significantly different mean ultimate strength at 210 $\pm$100 kPa from single layer albumin ($p = 0.3$).

t-test confirmed that the means for the single layer and double layer denatured albumin were not significantly different ($p = 0.3$).

The qualitative test on albumin concentration was performed after several soldering experiments which 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. We 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 $350 \pm 36\,\mathrm{J/cm^2}$ required to turn the albumin golden brown.

Liver repaired on the parenchymal (exterior) surface and on a non-parenchymal surface (interior) using the ABC and 38% albumin in two layers is presented in figure 5. 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. The remaining 7% (2 samples) failed at the superglue holding the liver to the aluminum plates and partially tore the albumin and so the samples were not included in either group. The mean tensile stress for tearing was 39 ± 13 kPa and 7.4 ± 3.8 kPa when the bond between the albumin and 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 ± 8 kPa and 5.9 ± 4.7 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.

4. DISCUSSION

Tensile loading of liver repaired with ABC and albumin solder failed in one of two methods regardless of the surface of the liver 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 of failure.

ABC soldering took a mean repair time of 5.8 ± 0.6 seconds per square centimeter at a power setting of 60 W to denature two layers of albumin corresponding for a mean total energy of $350 \pm 36\,\mathrm{J/cm^2}$ 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[8] thereby decreasing the time it takes to coagulate the albumin. Even so, ABC repair time was roughly twice as fast as the 12 ± 5 seconds per square centimeter for the laser welds achieved by Wadia et al..[9]

The qualitative assessment of albumin concentration showed that $38 \pm 2\%$ was the easiest to apply. Poppas et al.[12] 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. Without manual spreading, the 53% albumin would not denature completely through to the liver parenchyma. The highest concentration that did not require manual spreading was 40%. Use of 38% albumin eliminated operator error in deciding what constituted a thin enough layer to achieve an adequate repair and it also kept the sticky albumin off the gloves of the surgeon.

Coagulated 38% albumin also matched the ultimate tensile strength of native liver. The mean ultimate strength of 38% denatured albumin (in two layers) was comparable to healthy liver failing at 210 ± 100 kPa and 270 ± 220 kPa respectively. 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.

The ultimate tensile strength of coagulated albumin (25, 38 and 53%) in a single layer and two-layered denatured 38% albumin were not significantly different ($p = 0.3$). Two layers of albumin required more force to fail since the cross-sectional area of the solder was greater. ABC soldering with 38% albumin took $6\,\mathrm{s/cm^2}$ to coagulate two layers of solder and gave the best balance among ease of use, strength, and matching of ultimate tensile strength to that of the liver *in vitro*.

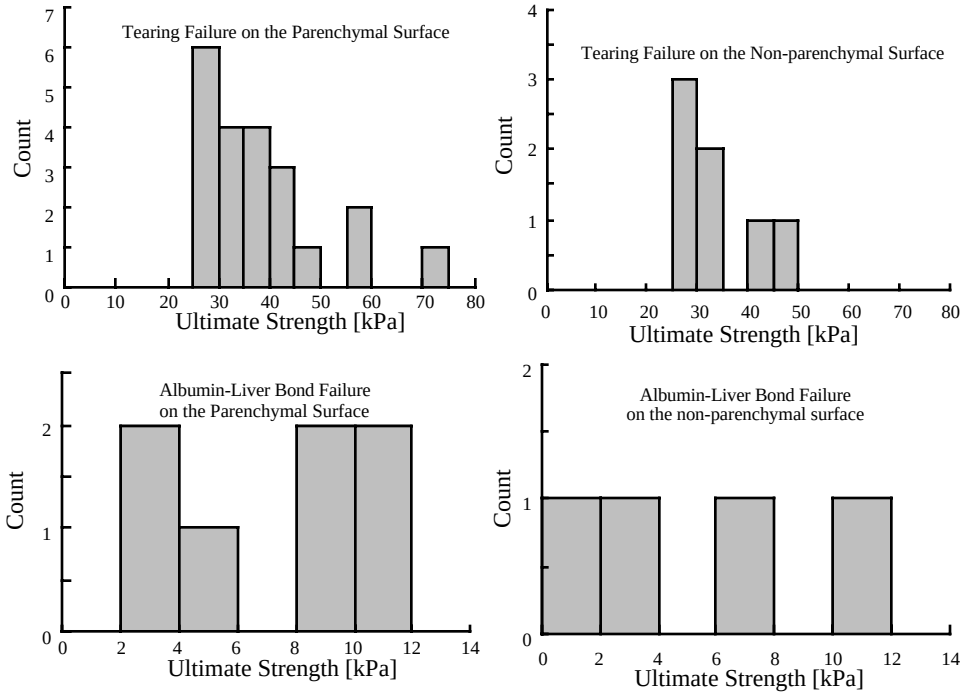

Figure 5. Repaired liver on the parenchyma or interior surface failed by one of two methods, the albumin tore along the incision or the albumin broke free from the liver when soldered with 38% albumin and loaded with tension at 2 mm/s.

5. ACKNOWLEDGEMENTS

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