Laser welding of biomaterials stained with indocyanine green to tissues.

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Abstract

This paper considers some issues pertinent to laser welding of elastin-based biomaterials to tissues using a pulsed diode laser (10-μs pulse) and indocyanine green (ICG) as an absorbing chromophore to localize laser heating to the "weld surface", the elastin/tissue interface where welding occurs. Experiments involved laser welding of elastin heterographs to the intimal surface of the carotid artery (in vitro, porcine) as a ~4x5 mm^2 spot weld, then determining the breaking strength when the two tissues were pulled in a direction parallel to the plane of the spot weld while submerged in water. The questions answered are:

• WHAT IS THE PEAK TEMPERATURE REQUIRED FOR WELDING ELASTIN HETEROGRAPH TO THE INTIMAL SURFACE OF CAROTID ARTERY?

ANSWER: ~300 °C threshold, ~600 °C for maximum strength. This estimate is based on optical measurements of dye accumulation in stain layer and measurements of thickness of stain layer via fluorescence microscope examination.

• WHAT IS THE DEPENDENCE OF WELD STRENGTH ON THE LASER EXPOSURE?

ANSWER: Breaking force (g) = Max*(1 - exp(-(E_p - E_{th})/U_{67})), where Max is the maximum strength achievable by laser welding, expressed as the breaking force in g when elastin heterograph and tissue are pulled. E_p is the laser pulse energy. E_{th} is the apparent threshold laser pulse energy that will break the weld. U_{67} is the laser energy above threshold which achieves 67% of Max. Max was about 15 g for the ~20 mm^2 weld area of our experiments. E_{th} was 0.8 J. U_{67} was 1.44 J.

• DOES WELD STRENGTH DEPEND ON HYDRATION CONDITIONS?

ANSWER: Not on the amount of excess unbound water. There was no significant difference in weld strength between welding dripping wet tissues vs well blotted tissues.

• WHAT DIFFERENCE IS THERE BETWEEN IRRADIATING THE WELD SURFACE THROUGH THE BIOMATERIAL VS THROUGH THE TISSUE, WHEN THE BIOMATERIAL IS PARTIALLY STAINED WITH ICG?

ANSWER: There is a difference if the stain layer is heavily stained. Irradiation through the tissue allows direct irradiation on the weld surface which achieves the highest peak temperatures for the least laser pulse energy. Irradiation through the elastin heterograph causes direct irradiation of the rear surface of the stain layer, within the biomaterial and away from the weld surface, and thermal diffusion must bring the heat to the weld surface. This difference occurs only when the absorption by the stain layer is sufficiently high that little laser energy directly reaches the weld surface.

1. INTRODUCTION

Elastin is an attractive biomaterial for creating artificial tissue for use in anastomosis of cut tissues or in relining the lumen of tubular structures such as blood vessels, ureters, esophagus, colon, etc. This paper reports early efforts to weld elastin heterographs to carotid artery lumen.

2. EXPERIMENTAL METHODS

2.1 Elastin heterographs

Carotid arteries from pigs were obtained freshly at slaughter. After dissection of the arteries, some were incubated at 65 °C for 1 hr in 5 M NaOH to dissolve all tissue components except the elastic lamina of the vessels. These elastin structures are called "heterographs". They were routinely stored in water. The one surface of the heterographs was exposed to a 0.1 M solution of indocyanine green (ICG) for 10 min, which achieved an ICG staining of the elastin surface that was shown under fluorescence microscopy to extend about 100 μm into the tissue.

2.2 Optical measurements of ICG staining

The amount of ICG that accumulated in the elastin heterograph was determined by an optical transmission measurement at 805 nm. A diode laser was introduced into an integrating sphere and a port left open for viewing by a 2-m distant CCD video camera, yielding a uniform backlighting for transillumination of elastin samples that were placed on the port. To calibrate the system, a set of nonscattering optical density filters were placed at the port and camera images acquired by computer. The mean pixel value of the transillumination images were determined using Image (NIH). The cross-plot of mean pixel value vs transmission, $T = 10^{-OD}$, was linear and served as a calibration curve. Hence, subsequent measurements that yielded a mean pixel value were converted into transmission, T.

A transmission measurement through an elastin sample that was unstained by ICG provided a baseline transmission where attenuation was simply due to scattering. A baseline optical depth ($b_{baseline}$) was calculated: $b_{baseline} = -\ln(T)$. Transmission measurements through the ICG-stained elastin samples were acquired and converted into optical depths:

$$b = -\ln(T)$$

The differences in optical depth were calculated, $b - b_{baseline}$, and attributed to the attenuation due to the ~100-μm-thick ICG layer in the elastin. Hence the absorption coefficient, μ_a [cm^{-1}], was estimated:

$$\mu_a = \frac{b - b_{baseline}}{0.0100\ cm}$$

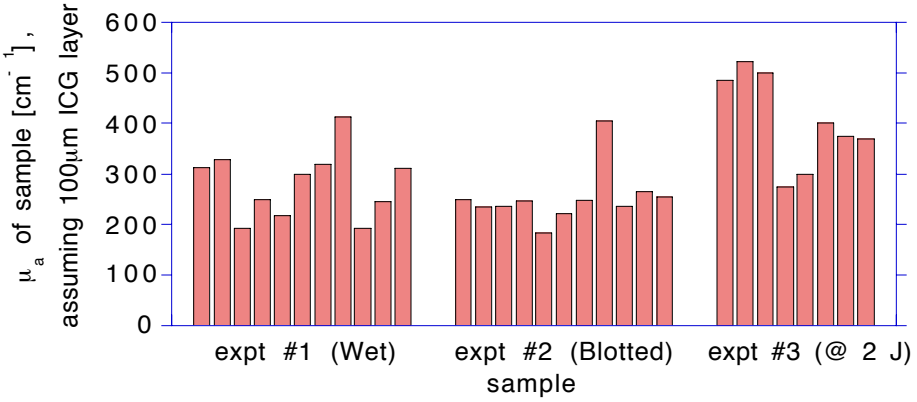

Figure 1: ICG staining of elastin heterographs. The cited absorption coefficient, μ_a [cm^{-1}], assumes ICG was uniformly distributed in the 100-μm thickness.

2.3 Laser welding

The elastin heterograph was cut length-wise and opened to yield a flat section of tissue about 200 μm thick, 6 mm wide, and 2 cm long. The elastin was placed ICG-stained side down onto the intimal surface of a porcine carotid artery that had also been cut and opened as a flat tissue section. This tissue composite was placed between glass microscope slides and held compressed under mild pressure exerted by a ring of 169 g mass that rested on the glass slides. Down into the center of the ring was inserted the laser delivery system comprised of a diode laser array and a conical beam guide with copper surfaces that concentrated the laser emission into a 6x6 mm^2 area. This device was placed in contact with the glass slide over the site where elasin heterograph overlaid the carotid surface. Ten laser pulses were delivered at 1 Hz, yielding a weld between elasin and carotid. The welded tissues were returned to a vial of saline.

In some tests, the elastin and carotid tissues were dripping wet at the time of welding. In other tests, the elastin and carotid tissues had been well blotted with tissue paper before welding.

2.4 Stress/strain experiments

The stress/strain experiments involved placing the welded elastin/carotid specimens in a device that pulled the tissues apart while recording stress and strain. The free ends of elastin and carotid were held by grips on the device. The pulling was tangential to the plane of the spot weld. The tissue was pulled apart while under water.

Figures 3 and 4 shows the raw data generated by the stress/strain experiments. Weld strengths were assessed by the load (g) that achieved breakage of the weld. The slope of the stress/strain curve in Fig. 3 is 0.00129 g/μm, or 64 g/cm^3 when normalized by the ~20 mm^2 area of welds in these experiments and expressed in cgs units. The weld area was observed to be less than the 36 mm^2 area of laser exposure and further work on resolving this discrepancy is planned.

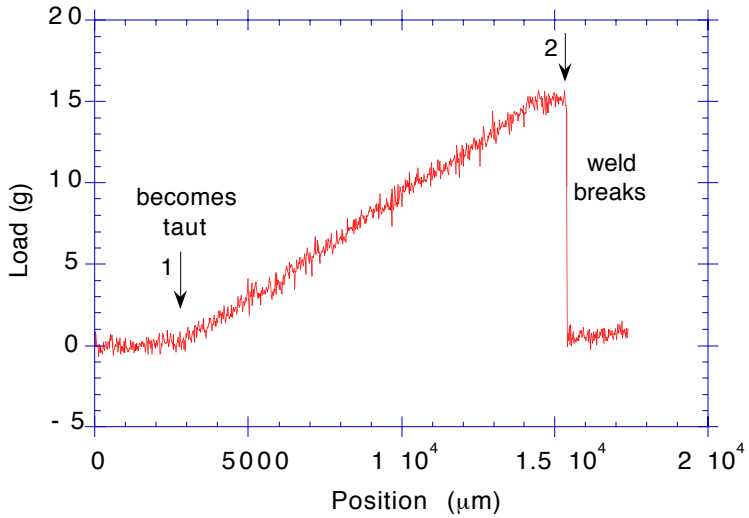

Figure 3: Stress-strain curves for elastin/carotid welds. Curves show original data expressed as load (g) vs position (μm). Area of welds was ~20 mm^2. Stress ≈ load/(20 mm^2). Shows the curve for strongest weld made with 4.35-J laser pulse. Arrow 1 indicates the beginning of stress when welded tissue becomes taut. Strain = position - (Arrow 1). Increasing strain yields increasing stress (stress/strain ≈ 0.0064 g/cm^2). Arrow 2 indicates when weld broke.

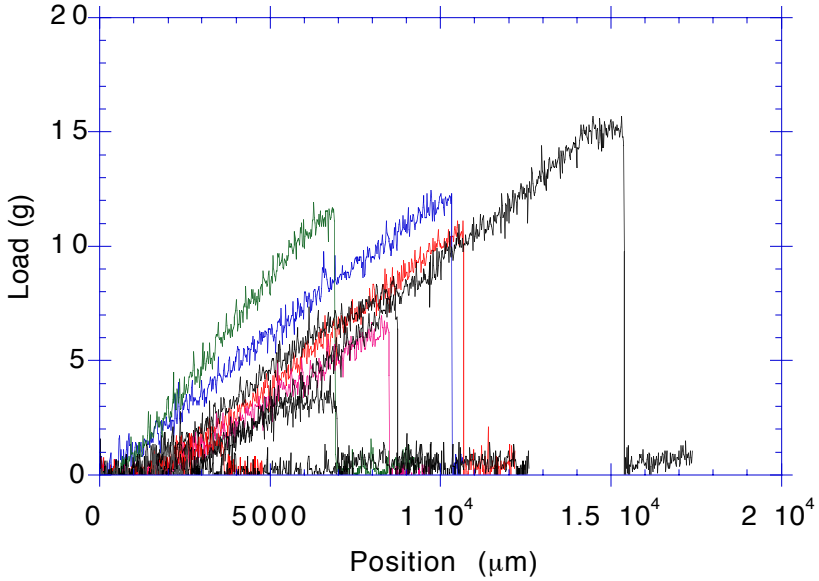

Figure 4: Stress-strain curves for elastin/carotid welds. Shows the curves for a set of welds created using different laser pulse energies. Weld made with lower energies broke at lower loads.

3. RESULTS

3.1 Weld strength vs laser pulse energy

Three experiments were conducted: (1) tissues dripping wet, (2) tissues well blotted, (3) repeat of dripping wet vs well blotted. Figure 5 shows the results of experiments 1 and 2. Figure 6 shows the results of experiment 3.

The experimental results are summarized in Fig. 4. The breaking force [g] is plotted vs the laser pulse energy [J]. The weld strength is characterized by the breaking force which increased with increasing pulse energy, exponentially approaching a maximum strength. Above about 4 J, the maximum weld strength was attained.

In experiment 3, both dripping wet and well blotted specimens were welded using 2 J of energy. There was no significant difference in the weld strength whether the elastin and carotid specimens were dripping wet at welding or had been well blotted.

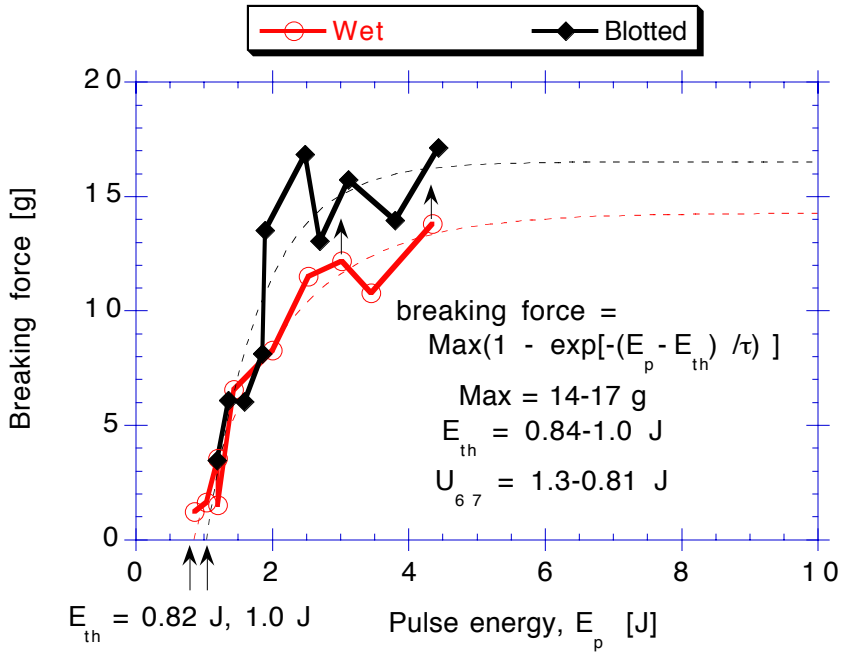

Figure 5: Weld strength vs laser pulse energy. The weld strength is expressed as the load (g) at which the weld broke. The wet and blotted tissues were not significantly different in their behavior. (The two arrows indicate two welds where the heterograph broke before the weld broke, and hence these data are lower-limit values for the breaking force.)

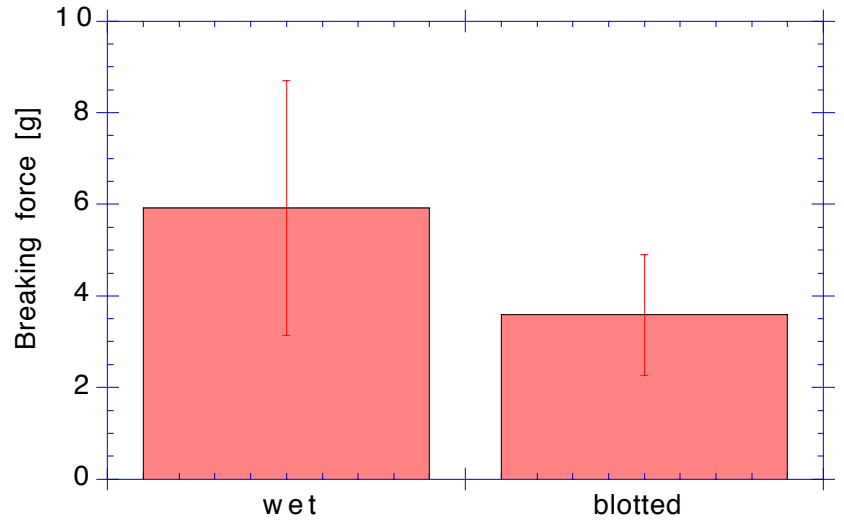

Figure 6: There was no significant difference between welding specimens that were dripping wet versus well blotted. Laser pulse enegy was 2 J for this experiment 3. The slight differences in weld strength amongst tested specimens were more due to variation in ICG staining than due to wet vs blotted (see Figs. 7 and 8).

3.2 Temperature required for welding

The temperature jump at the ICG layer could be estimated by multiplying the absorption coefficient of the ICG layer (μ_a, see Fig. 2) by the laser pulse energy (E_p) and dividing by the area of the irradiation (Area = 24 mm^2) and by the product of density and heat capacity ($\rho C \approx 3.8$ °C/(J/cm^3)):

$$\Delta T = \frac{\mu_a E_p}{\text{Area } \rho C}$$

Figure 7 shows the breaking force as a function of calculated temperature jump. for the 3 experiments. Welding begins when ΔT exceeds about 300 °C, and increases with increasing ΔT toward a maximum strength achieved by about 600 °C.

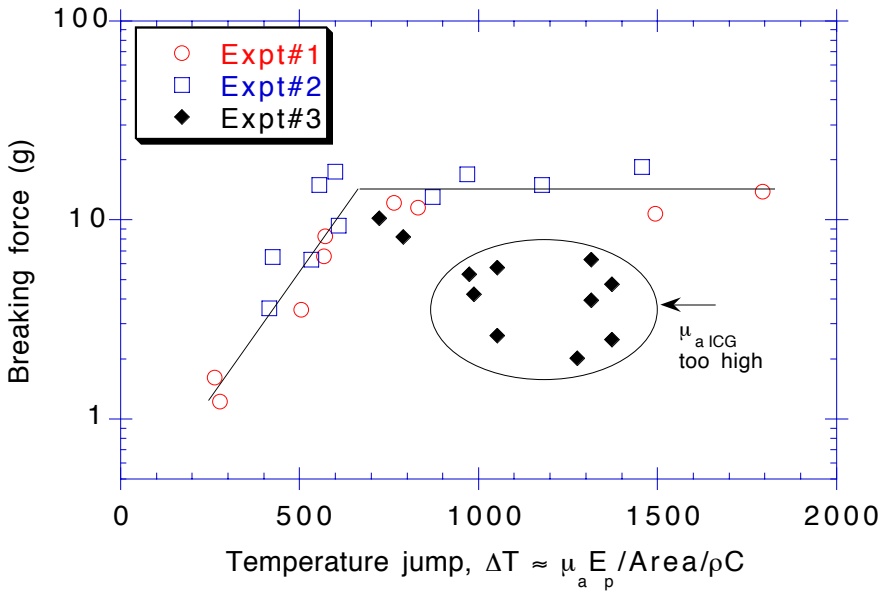

Figure 7: Weld strength increases with the peak temperature achieved by laser exposure. Breaking force of weld is plotted vs calculated temperature jump.

Some of the data from Expt. 3 deviated from the general pattern of weld strength increasing with ΔT (9 data diamond points within oval). The deviant data points corresponded to elastin heterographs which had higher than normal levels of ICG in the stain layer. Figure 8 shows how weld strength decreases when the ICG staining is too strong. Because the laser must transmit through the stain layer of the elastin heterograph before reaching the weld surface, too high an absorption inhibits the heating of the weld surface.

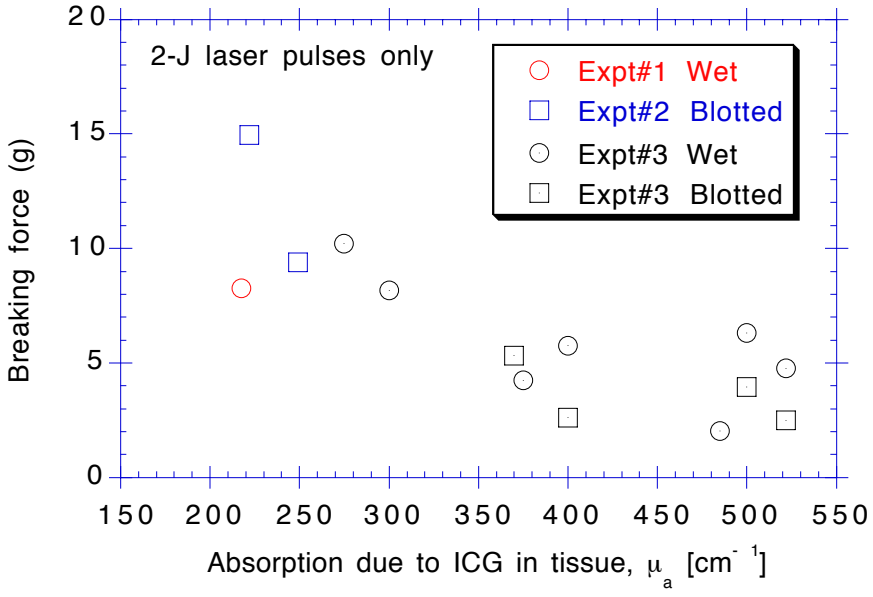

Figure 8: Weld strength decreases when the ICG staining was too high. The breaking force is plotted vs absorption coefficient of the ICG layer. Data shown are only those experiments involving 2-J laser pulses.

5. DISCUSSION

The rather high temperature required for the onset of welding (~300 °C) is much higher than required for coagulation of most proteins. However, elastin is an especially heat-resistant material. It is possible that this high temperature is required for the short transient time of heating to achieve melting of the elastin.

The weld strength saturated when the ΔT reached about 600 °C (apparent ΔT; better to discuss as an increase in energy density of 2280 J/cm^3). This is close to the energy density required for the enthalpy of water vaporization. Possibly, ΔT jumps above 600 °C simply vaporize water rather than heat the tissue, and therefore no further increase in weld strength is seen. Another possibility is that weld strengths simply can't exceed this saturated value.

Irradiating through the elastin heterograph involves heating the back side of the ICG layer, still within the elastin, rather than directly heating the elastin/carotid interface. Irradiating through the tissue (carotid) allows the light to first strike ICG at the weld surface. The latter approach should be better. We chose to irradiate through the heterograph in these early experiments because it was simpler to handle the tissues for exposure through the heterograph. Transmission through the carotid is about 80% at 805 nm, however, and this may be the better way to execute welding.

6. CONCLUSIONS

• The peak temperature required for welding elastin heterograph to intimal surface of carotid artery is ~300 °C threshold, ~600 °C for maximum strength.

• The weld strength depends on the laser pulse energy:

$$\text{Breaking force (g)} = \text{Max}*(1 - \exp(-(E_p - E_{th})/U_{67}))$$

The value Max was about 15 g for the ~20 mm^2 weld area of our experiments. E_{th} was 0.8 J. U_{67} was 1.44 J.

• Weld strength does not depend on the amount of excess unbound water. There is no significant difference in weld strength between welding dripping wet tissues vs well blotted tissues.

• There is a difference between irradiating the weld surface through the biomaterial vs through the tissue, when the biomaterial□\s□pUqtUUlluTstUqUUdXwqtUTECG□P WqtTDlqWTt□UCG□stUiUinW, lqradiationDtTrot◊TPthU UlasqUTwas nWt `TproblUll but witT heavy UCG staining tTUTexdUss□UCG vlowked the l]ghtPdeliuUr}Tto8thuTelastimØcarotid interface. Irradiation through the tissue (capotid) is an alternative method which should directly heat the elastin/carotid interface and achieve maximal temperatures.

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∑. ACKNOWLEDGEMENT

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