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A Reconstituted In Vitro Clot Model for Evaluating Laser Thrombolysis

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Abstract. Background/Objective: **Laser thrombolysis is the selective removal of thrombus from occluded blood vessels using laser energy. A reconstituted clot model with reproducible optical absorption properties was developed to evaluate the effect of various laser parameters on thrombus removal rate.**

Study Design/Materials and Methods: **Reconstituted clots were made with known fibrinogen concentrations and hematocrits. *Ex vivo* clots were collected from ten swine. Four red gelatin phantoms were prepared. Mass removal rates and ablation efficiencies were determined using a 577 nm, 1 μsec pulsed dye laser. The ablation efficiencies of the three clot models were compared at an energy of 25 mJ and a repetition rate of 4 Hz. In addition, the reconstituted clot model was ablated as pulse energy and repetition rate were varied with average power held constant at 100 mW.**

Results: **The mean ablation efficiency for *ex vivo* clots ranged from 0.4 ± 0.1 to 3.4 ± 0.7 μg/mJ/pulse, with significant differences between groups (ANOVA $p < 0.05$). Reconstituted clots of varied fibrinogen content had ablation efficiencies of 1.5 ± 0.2 to 1.6 ± 0.3 μg/mJ/pulse at this energy and repetition rate. Gelatin ablation efficiency was inversely proportional to protein content and ranged from 0.5 ± 0.3 to 2.0 ± 0.7 μg/mJ/pulse. Reconstituted clot mass removal rates (in μg/s) were clinically similar for settings ranging from 13 mJ at 8 Hz to 33 mJ at 3 Hz.**

Conclusions: **The reconstituted model clot is a reproducible and biologically relevant thrombolysis target. *Ex vivo* clot lacks reproducibility between individuals and gelatin phantoms lack clinical relevance. At a constant average power, varying laser parameters did not affect mass removal rates to a clinically significant degree.**

Key Words. **ablation, mass removal rate, stroke**

Introduction

Laser thrombolysis is the photomechanical removal of thrombus. Pulsed laser energy is absorbed by the hemoglobin pigment in the clot, causing the formation of a cavitation bubble. The collapse of this bubble mechanically disrupts and eventually removes the clot [1,2]. Earlier work utilizing laser thrombolysis for the treatment of acute myocardial infarction [3–5] and pre-clinical studies in a swine cerebral thromboemboli model [6] indicate that this therapy is a viable, selective, and safe method for the recanalization of occluded cerebral arteries. This study develops and validates a new reconstituted clot model that is more physiologically relevant than previous gelatin clot models used in laser ablation studies [7]. This clot model was used to test five sets of laser parameters, having equal average power.

Laser thrombolysis for acute stroke therapy is currently being tested in a clinical trial [8]. The laser thrombolysis system delivers laser energy to the clot through a flexible fluid core catheter [9]. The laser is coupled into a fused silica fiber, which carries the laser energy to nearly the end of the catheter. Radiopaque contrast solution is continuously injected through the catheter. After the laser light exits the fiber it is transmitted through the optically clear contrast solution to the occluding thrombus with a spot size of approximately 0.8 mm^2 [10]. The contrast solution is atraumatic, angiographically visible, removes ablated thrombus particles, and convectively cools the area. Because the fiber terminates before the end of the catheter, potentially dangerous contact with arterial tissue is prevented.

Hemoglobin is the primary absorbing chromophore in thrombus at wavelengths ranging from 400–590 nm. Within this spectrum, light selectively ablates thrombus and not vascular tissue [11]. The catheter system tested uses a 1-μsec pulsed dye laser emitting at 577 nm (Palomar 3010, Beverly MA). This pulse duration is much less than the time required for thermal confinement [11]. At 577 nm, thrombus has a much lower ablation threshold (0.02–0.03 J/mm^2) than the damage threshold for vessel tissue (1.1 J/mm^2 in saline, 0.16 J/mm^2 in blood) [11]. The ablative event is due to the formation and rapid collapse of a cavitation bubble, the force of which increases with energy [1]. In the confined space of a cerebral artery, the force of this bubble collapse becomes a potential safety concern. It may be possible to increase the safety margin of this therapy without compromising efficiency of clot removal by lowering

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the energy level and proportionally increasing the repetition rate [12].

For this study, a new *in vitro* model clot was developed to test the effects of varying pulse energy and repetition rate on the ablation of thrombus. Previous investigations have successfully measured the effects of altering laser parameters on red-dye gelatin phantoms [1,7], but the clinical relevance of a non-biological clot target is questionable. *Ex vivo* thrombus (static clot), formed by allowing whole blood to clot in tubing, has been used for thrombolysis [2,7,13–18]. This model clot lacks reproducibility, due to variations between donor individuals in hemoglobin concentration (hematocrit) and fibrinogen concentration.

The reconstituted clot model was developed to provide a simple, reproducible yet clinically relevant clot target in which the hemoglobin and fibrinogen concentrations were controlled. The fibrinogen concentration has previously been demonstrated to be directly proportional to the mechanical strength of the clot [19]. The mechanical properties of the model clots are the result of enzymatic reactions that mimic those found in the final common pathway of the clotting cascade *in vivo*. In short, fibrinogen is added to whole blood, then converted to fibrin by thrombin. It is important to note that this model does not take into account additional biochemical events that occur during clotting *in vivo*. These include fibrin-fibrin crosslinking by fibrin stabilizing factor (Factor XIII) [20] and the clot contraction through the action of platelets. Extensive remodeling of the thrombus also occurs intravascularly over time through the action of plasmin. Characterization and optimization of these phenomena were outside the scope of this study.

The effects of alterations in laser energy and repetition rate on ablation rate (μg/s) were assessed using fibrinogen concentrations of 300 mg/dL. The normal range of fibrinogen in swine is 100–500 mg/dL [21], and 200–400 mg/dL in human plasma. Therefore, the 300 mg/dL reconstituted model clot represents a median concentration for both human and swine thrombus. The majority of thrombotic emboli in ischemic stroke are caused by atrial fibrillation [22]. Freshly formed, this clot typically has approximately the same amount of hemoglobin containing erythrocytes as found in whole blood [23]. The reconstituted clot model most closely models thrombus of this type.

The goals of this study were to (1) develop a reproducible clot model using native blood components and to (2) compare the reproducibility of this reconstituted clot to that of static and gelatin clot models, (3) use scanning electron microscopy (SEM) to investigate the structural differences between the static and reconstituted model clots, and (4) test the effects of various laser parameters on ablation rates of a reconstituted model clot at an average power of approximately 100 mW as pulse energy was decreased and repetition rate was proportionally increased.

Materials and Methods

Thrombus models

Static clot

Whole blood was drawn from domestic swine into a 25–35 cc syringe with an 18G needle and immediately injected into (2.5 mm ID) IV tubing (Baxter Healthcare Corp., Deerfield IL). The tubing was folded in half and suspended for 12–24 hours at room temperature. Hematocrit was measured for each animal, and ranged from 25.3–33.8%. Six preparations were ablated within 24 hrs of collection and 4 were ablated at 96 hrs. The 96 hr. blood samples were analyzed for fibrinogen concentration (Beckman Electra 1600C Coagulation Analyzer). This procedure is adapted from the method for forming static clots previously reported by other investigators [2,7,13–18].

Reconstituted clot A

Whole blood from domestic swine was collected in citrated blood donor bags (CPDA1, Baxter Healthcare Corp.) and centrifuged at 2280 $\times$ g for 20 min. at 4°C. The plasma supernatant was frozen at -70°C for at least 24 hr and slowly thawed at 4°C. The cryoprecipitate, which contains most of the fibrinogen [24] was removed. Porcine fibrinogen (Fraction I, Sigma, St. Louis MO) was added to a concentration of 300 mg/dL plasma. The erythrocytes were mixed with Adsol preservative (Baxter Healthcare Corp.) and stored up to 30 days. Prior to recombination, this red blood cell preservative was removed by centrifuging at 1000 $\times$ g for 5 min. at 4°C. The separated erythrocytes and plasma were then recombined to a hematocrit of 40–45%, the normal human range. To form the reconstituted clot, 250 US units of bovine thrombin (Jones Pharma, Middleton WI) in 1 mL Tris buffered saline (TBS) with 5 mM $CaCl_2$ was drawn into a 35 mL syringe followed by 34 mL of whole blood. This mixture was immediately injected into 2.5 mm inner diameter IV drip tubing (Baxter) and then incubated in a 37°C water bath for 1 hour.

Reconstituted clot B

Whole blood from swine was collected in CPDA1 donor bags (Baxter) and centrifuged at a relative centrifugal force of 2100 $\times$ g for 30 min. at 4°C. The plasma supernatant was heated to 53–56°C for 3 min., causing the fibrinogen to precipitate from solution [14]. Plasma fibrinogen was measured and determined to be $<$60 mg/dL (Electra 1600C Coagulation Analyzer, Beckman). Porcine fibrinogen (Sigma, Fraction I) was added to a concentration of 300, 600 or 1,200 mg/dL. The separated erythrocytes and plasma were then recombined to a hematocrit of 28%. To form the reconstituted clot, 1,000 US units of

thrombin (bovine, Jones Pharma) (in 1 mL TBS with 40 mM $CaCl_2$) was drawn into a 35 mL syringe followed by 34 mL of whole blood. This mixture was immediately injected into IV drip tubing and then incubated in a 37°C water bath for 1 hour.

All procedures used in this study were conducted in accordance with institutional guidelines at Oregon Health and Science University concerning the care and use of experimental animals.

Gelatin phantom

300 Bloom Gelatin (Sigma) was mixed with a 0.18% aqueous solution of Direct Red 81 dye (Sigma) in proportions of 5, 10, 15, and 20% gelatin (wt/wt). The mixtures were allowed to soak for 4 hr, then heated to 65°C for 25 min. The solutions were injected into IV tubing (Baxter, 2.5 mm ID). The samples were allowed to cure in a 10°C water bath for 18 hr. prior to testing. These methods were adapted from gelatin bloom strength measurement standards [25].

Scanning electron microscopy

Samples of the static and reconstituted model A clots were fixed in 4% glutaraldehyde ≥12 hr, then rinsed with phosphate buffered saline (PBS). The samples were serially dehydrated with increasing concentrations of ethanol, then exchanged into increasing concentrations of Amyl acetate (Sigma). The Amyl acetate was removed with liquid CO_2 in a critical point dryer (CPD2, Pelco International, Redding CA). Samples were anchored to aluminum posts using colloidal silver paint (Ted Pella Inc., Redding CA) and sputter coated in a Hummer IV Sputtering System (Technics Corp., Alexandria, VA). Scanning electron microscopy was performed in an Amray 1810 SEM.

Laser ablation

The laser thrombolysis (ablation) experiments were performed with a Palomar 3010 pulsed dye laser emitting a 1-μsec pulse at 577 nm. Energy was measured before and after each experimental set (EM400, Molectron, Portland OR). The average value of these two measurements was used as the effective laser pulse energy. For all the ablation experiments, the average power was approximately 100 mW for 30 seconds. These parameters mimic those in the current clinical trial. To determine the effects of decreasing pulse energy while proportionally increasing repetition rate, the effective energy was 12.6, 14.7, 20, 24, and 32.8 mJ with corresponding repetition rates of 8, 7, 5, 4, and 3 Hz, respectively.

A fluid core catheter (approximately 1.0 mm ID) and a 200 μm fused silica fiber (SpecTran, Avon CT) were used in this study. The fluid core catheter acts as a conduit for the radiopaque contrast dye (Hypaque 60, Nycomed, Atlanta GA) and the fiber. The contrast dye was injected at a rate of 4.2 mL/min. Laser energy is transmitted through this fluid to the occluding clot. The catheter was positioned within 1–3 mm of the model clot. This distance from the proximal surface was maintained as the target was ablated.

A 3 cm section of tubing was cut and the model clot was released into phosphate buffered saline (PBS). The clot was drawn into a 6.5 cm long section of Silastic silicone tubing (Dow Corning, Midland MI) with a 3.4 mm inner diameter and a 4.7 mm outer diameter. This inner diameter corresponds to that of vessels encountered in the cerebral circulation targeted for clinical application. The Silastic tubing with model clot was fixed in an ablation holder which had a diverting piece of tubing that allows flow of contrast solution and ablated particles from the lumen of the tubing (see Fig. 1). The fluid core catheter was advanced into the tubing containing the model clot until the catheter tip was 1–2 mm from the proximal face of the target. During ablation, the catheter was manually kept within 3 mm of the model clot. Contrast solution at 37°C was injected for 30 s to build

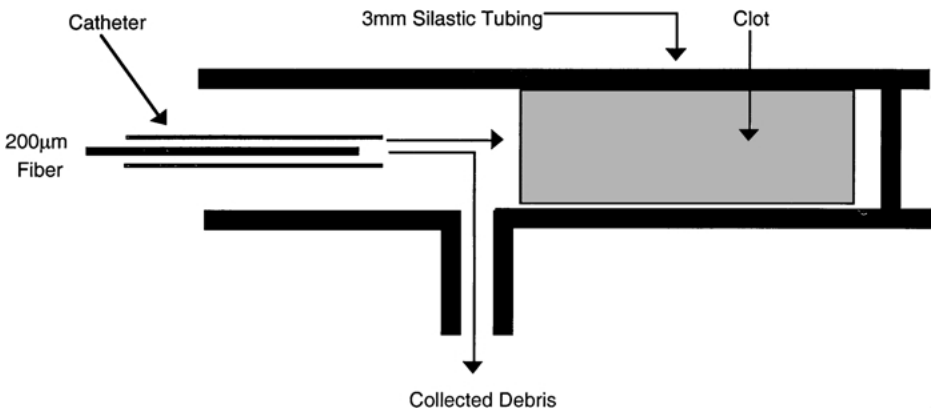

Fig. 1. *The in vitro laser thrombolysis experimental set up. Laser energy is delivered through the fluid core catheter to the clot in the ablation chamber. The ablation chamber contains a 3 mm inner diameter section of tubing which holds the clot and has a bypass for the collection of effluent.*

up to a 4.2 mL/min flow rate. Laser firing was performed for 30 s with simultaneous contrast injection. Following ablation, the proximal tubing was flushed with deionized water to collect all ablated fragments. Effluent was collected during the entire experiment and flushing. Deionized water was added to each effluent sample to bring the total volume to 10 mL. The effluent for both static and reconstituted clot ablations contained small fragments that were mechanically crushed. Intact erythrocytes were osmotically lysed in the hypotonic solution. Effluent samples from the gelatin ablations were slightly heated to liberate the dye. Control experiments with contrast flow but no energy were performed at the end of each experiment ($N = 3$) to account for mass removal due to non-ablative mechanical forces and fluid flow from the laser thrombolysis catheter system.

The absorbance of a solution at a given wavelength is directly proportional to its hemoglobin concentration. When the total volume is known, the mass of absorber is readily obtained from the concentration. The relationship is summarized in the following equation:

$$\text{Mass ablated } (g) = \frac{\text{Absorbance (410 nm)} - \text{Absorbance (800 nm)}}{k}$$

where the constant k is experimentally determined and is equal to the slope of the graph of absorbance difference versus mass. The hemoglobin in the reconstituted thrombus absorbs strongly at 410 nm; this wavelength provides the necessary sensitivity at the low concentrations of dissolved hemoglobin found in ablated samples. Direct Red dye from the gelatin samples absorbs strongly at 510 nm. There is minimal absorbance by hemoglobin and Direct Red at 800 nm, therefore this wavelength was used to correct for variation in the plastic cuvettes used in the experiments. This method was adapted from the method of Sathyam et al. (1996). To generate a calibration curve, a range of clot or gelatin fragments similar in mass to those that would be produced in the experiment were blotted for 10 sec on filter paper (Qualitative P8, Fisher Scientific, Pittsburg PA) and weighed to the nearest 0.1 mg in 50 mL beakers. 10 mL of deionized water was added to each sample and the clot was manually crushed and osmotically lysed. A 3 mL aliquot from each sample was measured using a spectrophotometer (HP 8425 Diode Array). Absorbance was measured at 410 nm and 800 nm for clot and 510 nm and 800 nm for gelatin, and the difference was plotted against mass (For an example, see Fig. 2). The slope of a linear curve fit is the calibration constant k (in g^{-1}). A new calibration curve was generated for every static and reconstituted model clot preparation. A single calibration curve was generated for the four gelatin phantoms which all contained the same dye concentration. The

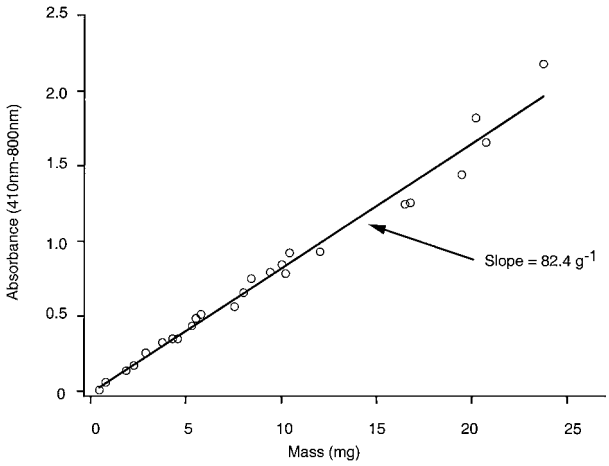

Fig. 2. *Calibration curve. Absorbance difference between 410 nm and 800 nm vs., mass (g), slope (k) = 82.4 g^{-1}. Each clot model formulation had its own calibration curve value. Absorbance values were measured at 510 nm and 800 nm for gelatin phantoms. The r^2 value for all linear regressions was $\geq$0.90.*

absorbance values were divided by the constant (k) to determine the total mass ablated during each 30 sec experiment. This calculation gives the mass removal rate in μg/sec. The ablation efficiency (μg/mJ/pulse) is calculated by dividing this value by the laser pulse energy (in mJ) and repetition rate (in Hz).

Mass removal rates at ~100 mW

Table 1 summarizes the experimental models utilized in each experiment. Static clot was collected from 10 domestic swine for the purpose of comparing the reproducibility of this model to the newly developed reconstituted clot model. Six of the static clot samples were ablated within 24 hrs. of collection and 4 more static clot samples were ablated 96 hrs. after collection. To measure the mass removal due to mechanical disruption by the fluid flow of the catheter system, 3 control (flow only) samples were tested from each clot. For the 24 hr. old clots, 6 samples were ablated from each, while 10 samples were ablated from each of the 96 hr old static clots.

Reconstitituted clot B was made from the recombination of plasma and cells from 4 animals. Fibrinogen concentrations were 300, 600, and 1,200 mg/dL Hematocrit was 28% for all three clots. Gelatin samples (5, 10, 15, and 20%) with Direct Red dye were also made. Ten samples of each of the reconstituted clot and gelatin phantoms were ablated, with 3 contrast flow-only controls. The model clots were ablated at an energy of approximately 25 mJ and a repetition rate of 4 Hz as described above.

The 300 mg/dL reconstituted clot A model was used in the increased energy (12.6, 14.7, 20, 24, and 32.8 mJ) and decreased repetition rate (8, 7, 5, 4, and 3 Hz) experiments. Average power was

Table 1. *Experimental Thrombus Models. Fibrinogen Concentration is Expressed in mg/dL Plasma*

Model	Age	N	Fibrinogen	Hct (%)	Clots/sample	Controls	Experiment
Static	24 h	36		25–34	6	3	Mass removal
Static	96 h	40	200–309	25–34	10	3	Mass removal
Reconst A	24 h	100	300	43	20	3	Energy/Rep rate
Reconst B	24 h	30	300–1,200	28	10	20	Mass removal
Gelatin	24 h	40			10	3	Mass removal

approximately constant (100 mW). Twenty experiments were performed at each setting and twenty contrast flow-only controls were performed. The reconstituted clot samples in this set of experiments all came from the same preparation. Hematocrit was 43%.

Statistical Analysis

Reported ablation rates and ablation efficiencies values are means ± 1 standard deviation. Significance of difference was determined by one-way analysis of variance (ANOVA) and t-tests using SPSS (Version 10.0, Chicago IL). Significance was defined as $p < 0.05$.

Results

Scanning electron microscopy

The ultrastructure of the static clot appeared to be more complex and the clot matrix to be more heterogeneous in size than in the reconstituted 300 mg/dL model clot (Fig. 3). The reconstituted clots exhibited fibrin fibers of uniform diameter (approximately 300 nm). Spheroid morphology (rather than the physiological biconcave shape) of erythrocytes was observed in all SEM prepared samples, due to the glutaraldehyde fixation and/or dehydration.

Mass removal rates at ~100 mW

The results of the ablation experiments are summarized in Figures 4–6. The mass removal rate (μg/mJ/pulse) experiments comparing the static, reconstituted, and gelatin clot models are summarized in Figures 4 and 5. For clarity, the ablated samples in Figures 4 and 5 have the model-appropriate control values (contrast flow only) rate subtracted. The mean control mass removal rates ranged from 4–65%, 2–23%, 4–9%, and 30–57% of the mean ablated mass for the 24 h static clots (1–6), 96 h static clots (A–D), reconstituted clots, and gelatin models, respectively. The 24 h static clots varied significantly (ANOVA, $F = 6.327$, $p < 0.001$) in ablation efficiency, as did the 96 h static clots (ANOVA, $F = 66.782$, $p < 0.001$). The age of the static clot (24 versus 96 h) significantly affected ablation efficiency (2 tailed t-test, $p < 0.001$). The blood donors utilized for static clots ablated at 96 hrs (Samples A–D, Fig. 4) were tested for fibrinogen concentration. The concentrations were 200, 216, 264, and 309 mg/dL for samples A, B, C, & D, respectively. There was no significant difference in ablation efficiency between the reconstituted clots of varying fibrinogen concentration (ANOVA, $F = 0.701$, $p = 0.505$). The gelatin models (Fig. 5) varied significantly (ANOVA, $F = 21.261$, $p < 0.001$) in ablation efficiency, with significant differences between 5–10% and 15–20% protein concentrations (Tamhane's T2, $p < 0.05$).

Figure 6 shows the results from the decreased energy, increased repetition rate experiments. The control value is contrast flow only. There were significant differences in the mass removal rate (μg/s) among the groups (ANOVA, $F = 17.472$, $p < 0.001$). The level of difference detectable at this experimental power is considered in detail in the discussion. The highest mass removal rate (400 ± 50 μg/s) was achieved at 20 mJ, 5 Hz and the lowest rate (270 ± 60 μg/s) was seen both at 14.7 mJ, 7 Hz and 24 mJ, 4 Hz. The largest difference in mean mass rate was 130 μg/s, between 20 mJ, 5 Hz and 24 mJ, 4 Hz.

Discussion

The overall goal of this study was to develop and characterize a reproducible reconstituted clot model using native blood components, and to compare this new model clot to the more widely used static clot and gelatin phantoms. Another objective was to measure the effects of varying laser parameters on the mass removal rate (μg/sec) and ablation efficiency (μg/mJ/pulse) of laser thrombolysis at 577 nm.

The lack of reproducibility in the strength of static model clots as well as variance in the hematocrits between individuals motivated the development of the reconstituted clot model. The mass removal rate of the static model clots varied significantly among the clots from the six swine. The static clot model is variable and unpredictable, both in fibrinogen and hemoglobin concentration (hematocrit ranging from 25–34%), for accurate *in vitro* testing of laser thrombolysis parameters. Since the age of thrombus *in vivo* correlates with its optical properties [26] and mechanical properties [20], these results suggest that stroke clots of varying ages may be removed clinically with the laser parameters previously discussed, although older clots will be removed significantly more slowly.

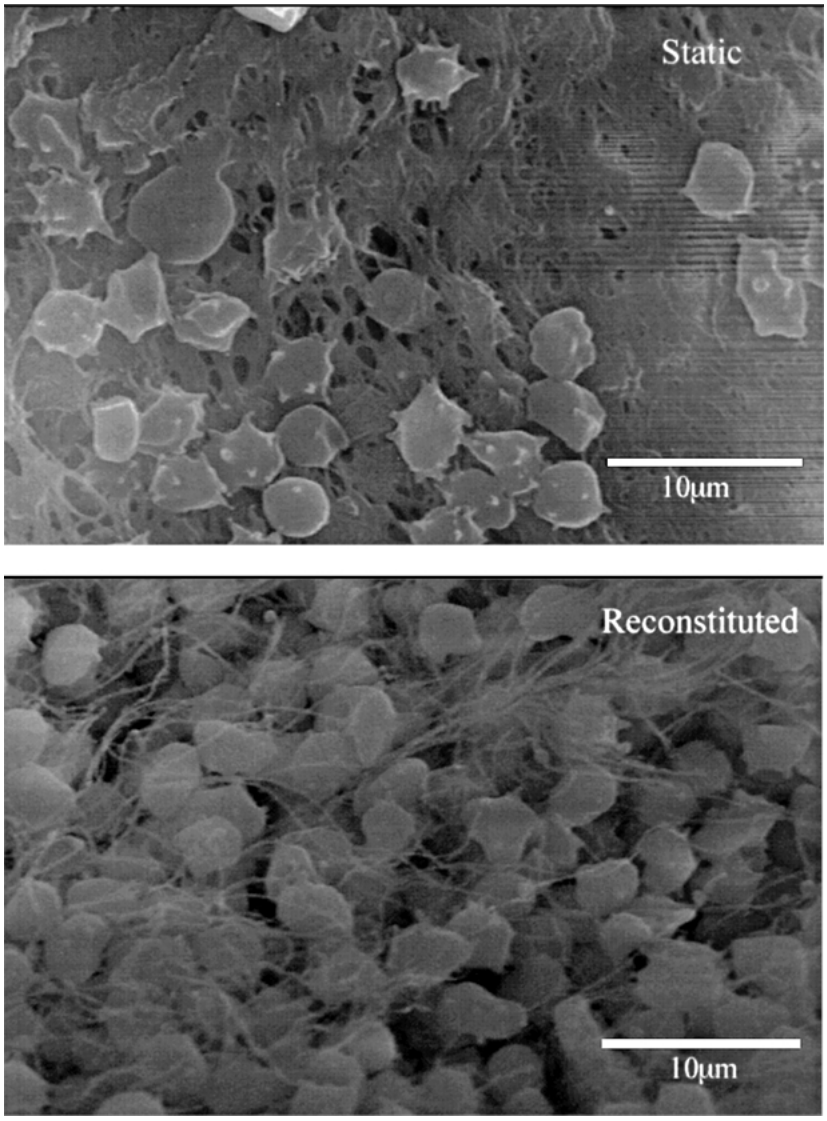

Fig. 3. *Scanning electron microscopy (SEM) of static and 300 mg/dL fibrinogen reconstituted model clots. The static clot was more heterogeneous than the reconstituted clot.*

Earlier gelatin phantom studies are consistent with our results, due to the homogeneity in strength and absorption coefficients between samples [7,13]. Detwiler (1991) used a similar gelatin phantom to measure the effect of unconfined compression modulus on ultrasonic thrombolysis. They concluded that ultrasonic ablation decreased with increasing protein content of their gelatin target [27]. These conclusions are supported by the current study of laser thrombolysis ablation of gelatin phantoms (see Fig. 5). Both ultrasonic angioplasty and the laser thrombolysis system under study remove target material through the formation and collapse of cavitation bubbles. The collapsing bubble generates a shock wave that is transmitted through the material, breaking it into smaller pieces. The differences demonstrated in the ablation of gelatin phantoms of varied protein content diminishes the value of gelatin as a thrombus phantom. Gelatin is a suspension of partially denatured collagen molecules, while clot is held together by a meshwork of fibrin. These structural differences appear to have an effect on the ablation of the models.

The ablation efficiency (μg/mJ/pulse) has previously been demonstrated to be independent of radiant exposure above threshold [1,2,7,11]. Previous experiments [12] with this reconstituted clot model and *in vitro* setup demonstrated no difference in ablation efficiency as the parameters of energy and repetition rate were varied with average power held constant at approximately 100 mW. The number of trials in this earlier study was chosen to be $N = 10$.

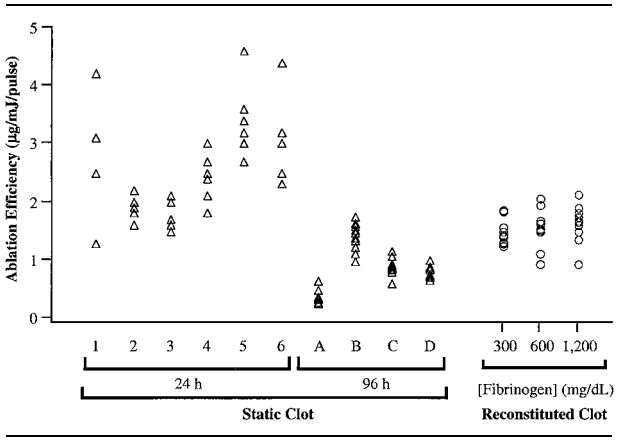

Fig. 4. *Ablation efficiency of the static and reconstituted clot models. Static clots are represented by open triangles, and each static clot category (1–6, A–D) represents clot from a different individual. Reconstituted clot is represented by open circles, and is reconstituted model B. There are significant differences among the static clot samples of each age group and between age groups (separate ANOVAs, $p < 0.001$). The reconstituted clot samples at 300, 600 and 1,200 mg/dL fibrinogen did not have statistically different ablation efficiencies.*

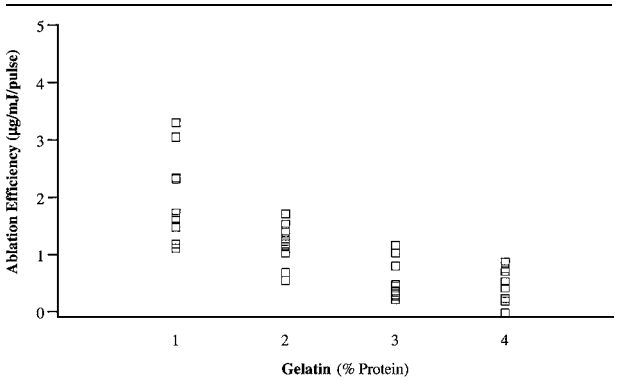

Fig. 5. *Mean ablation efficiency of the gelatin clot models. The gelatin models varied significantly (ANOVA, $p < 0.001$) in ablation efficiency, with significant differences between 5–10% and 15–20% protein concentrations.*

For the present experiments, a power calculation was performed using the previous investigation [12] as preliminary data. With $N = 20$, this study had 85% power to detect differences of at least standard deviations between groups. At this extremely high level of sensitivity, significant differences were demonstrated between most groups. The largest difference in mean mass removal was only 130 μg/s. The highest mean mass removal rate observed (at 20 mJ, 5 Hz) was 400 $\pm$ 50 μg/sec, while the lowest (at 24 mJ, 4 Hz) was 270 $\pm$ 60 μg/sec. With the sample size used in these experiments, the detectable difference (less than 0.2 standard deviation) in clot removal rates would translate to less than 2 μg/sec, which is too small to be clinically significant. These results

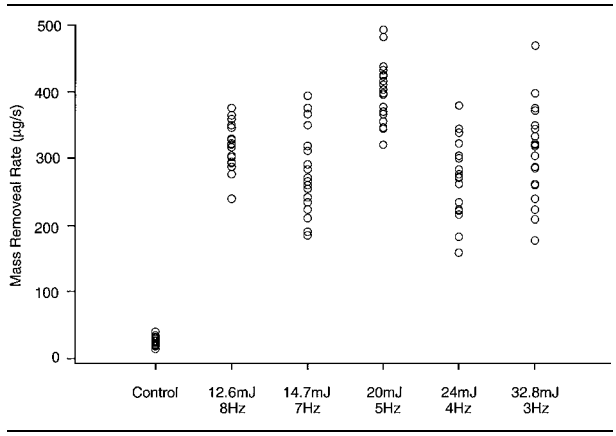

Fig. 6. *Mass removal rate of reconstituted model A clot at varying laser pulse energy (mJ) and repetition rates (Hz). Average power was approximately 100 mW. There were statistically, but not clinically significant differences among the groups.*

demonstrate that the clot mass removal rate can be maintained as pulse energy is decreased, as long as average power is approximately constant at 100 mW. This would reduce the force generated by the collapse of the cavitation bubble, which in the confined space of a cerebral artery, may be a potential safety concern.

The differences in ablation efficiency between the 24 hr and 96 hr static clots may have been due to crosslinking by Factor XIII over the additional time. The reconstituted clot models A and B also demonstrated significantly different mean ablation rates ($p < 0.05$), with the model A reconstituted clot ablating at approximately twice the rate as that of model B. The fibrinogen concentration was equal for the 300 mg/dL clots, therefore the differences in ablation rate appear to be due to differences in thrombin or calcium concentration, both of which have been previously shown to affect clotting [28,29]. The reconstituted model B clots had similar ablation efficiencies across a range of fibrinogen concentrations. These results agree with previous studies that demonstrate equivalent clot removal for similar model clots tested for tensile strength [30] and resistance to compression [10].

In this study as well as earlier ablation studies of thrombus, the static model clot was variable [1,2,7]. Gelatin phantom studies were shown to be more consistent due to the homogeneity in strengths and absorption coefficients between samples [7]. The reconstituted clot model is a reproducible and more biologically relevant *in vitro* target for bench top studies of laser thrombolysis parameters. The mass removed during control (contrast flow only) experiments on the reconstituted clots resulted in the lowest variation among the models. This decreased the error in the measurement of ablated mass due to laser energy, and further supports the usefulness of this

model over the others. The reconstituted clot model has some differences from static clot formed *ex vivo*. This is to be expected from a simplified clot phantom that lacks platelets and the complexity of the full hemostatic complement of the coagulation cascade. The static clot is limited to the endogenous concentration of thrombin generated by the *in vivo* clotting cascade, while the reconstituted model clot polymerizes much more quickly, with an excess of thrombin.

Future optimization studies of laser thrombolysis are now possible using this clot model. The reconstituted clot model has predictable optical properties and is a more biologically representative thrombus phantom than red gelatin because it consists of blood components, making it an ideal clot target for *in vitro* and even *in vivo* studies of laser thrombolysis as well as other mechanical thrombolysis therapies.

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