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Visualization of Microsecond Laser Ablation of Porcine Clot and Gelatin Under a Clear Liquid

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ABSTRACT

Laser thrombolysis uses microsecond laser pulses to remove thrombus-blocked arteries in the heart and the brain. Rapidly expanding and collapsing vapor bubbles are formed upon absorption of the laser energy by the thrombus. The goal of this study was to visualize the process of ablation and assess the effects of pulse repetition rate. The differences between contact versus non-contact of the laser delivery device with the thrombus were also investigated. Initial experiments were conducted with a gel-based clot model confined in 3 mm inner diameter silicone tubes. Subsequent experiments used 24 hour old porcine blood clots. Laser pulses of 50 mJ pulse energy were delivered via a quartz fiber contained in a flushing catheter. Pulse repetition rates of 1 Hz, 3 Hz, and 6 Hz were used. Wavelengths of 506 nm and 577 nm were used to ablate clot. Bubble action was captured by flash photography using a CCD camera and recorded on video. The amount of material removed was measured using a spectrophotometric technique. Bubble action was similar on clot and the clot model. No significant differences in bubble action or mass removal were observed at the three pulse repetition rates and the two wavelengths. Contact between the catheter and the clot did not result in a pistoning effect of the catheter at the pulse energy used.

Keywords: thrombolysis, clot, bubble, ablation, pulse, repetition rate

1. INTRODUCTION

Laser thrombolysis uses microsecond laser pulses to remove thrombus-blocked arteries in the heart thereby restoring blood supply to cardiac tissue.[1–3] The absorption of the laser light by the thrombus leads to vaporization of part of the clot and the formation of a vapor bubble. The bubble expands and collapses rapidly resulting in further disruption of the thrombus.[4] The wavelengths used for laser thrombolysis are in the visible region between 400–600 nm where the absorption of light by thrombus is nearly two orders of magnitude higher than that of arterial tissue.[5,6] The energy required to ablate clot is therefore lower than that required to ablate artery.[6–8] This provides a therapeutic window for selective removal of clot without incurring damage to the artery.

We finished a parametric study to identify the optimal wavelength, pulse energy, and spot size for efficient ablation,[9] and in another study we investigated the formation and collapse of vapor bubbles leading to ablation.[10] Our parametric and visualization studies used gelatin as a clot model. This removed the biological variability of clots. One of the goals of this study is to validate some of the results of the parametric and visualization studies by comparing clot and gel ablation.

Laser thrombolysis currently uses repetition rates of 3 Hz. The vapor bubbles formed upon absorption of the laser energy have lifetimes of the order of hundreds of microseconds. This suggests that the efficiency of ablation should be relatively independent of repetition rate, since cavitation bubble dynamics are finished before the arrival of the next laser pulse.

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Early thrombolysis and angioplasty efforts used solid glass fibers to deliver the laser energy, and in a few cases arterial perforations and dissections were reported. These were suspected to be caused by a pistoning effect of the hard fiber when in direct contact with the clot. Current laser delivery is achieved by a fluid core catheter that considerably minimizes the danger posed to vascular systems by the edges of the delivery device.[11] However, the concern about a pistoning effect due to direct contact with the clot remained.

The primary goals of this study were to compare ablation of clot versus gel by visualizing the process. We also investigated the effect of delivering the laser pulses at different pulse repetition rates on the ablation process and mass removed. Finally, we addressed the concern regarding a pistoning effect when the delivery device is in contact with the clot.

Porcine clot and gel were confined in 3 mm inner diameter silicone tubes to simulate arteries. Laser pulses were delivered using a fluid catheter at pulse repetition rates of 1 Hz, 3 Hz, and 6 Hz. Vapor bubble formation was recorded using freeze-action flash photography. The total mass removed was measured to quantify ablation efficiency. The laser pulses were delivered with the catheter both in contact and non-contact modes. Both 506 nm and 577 nm were used to ablate clot to study the effects of wavelength on the ablation efficiency.

The results of our study show that bubble action is similar during ablation of clot and gelatin. No significant differences in the and mass removal were measured as a function of repetition rate for clot and gel. Contact did not result in pistoning. Both 506 nm and 577 nm were equally efficient in ablating clot.

2. MATERIALS AND METHODS

2.1. Porcine Clot and Gelatin

Fresh non-heparinized blood was collected from domestic swine and allowed to clot in a waterbath at 37°C for 24 hours. It was then cut into 0.5–1 cm pieces and confined in silicone tubes. The silicone tubes had an internal diameter of 3 mm and a wall thickness of 400 μm. The walls were clear to facilitate visualization of ablation.

The clot model was made with 3.5% by weight gelatin in water. A dye (Direct Red 81, Sigma) was added to the gelatin to control the absorption at the laser wavelength (506 nm). At 506 nm, 0.1 g of the dye in 100 ml gelatin solution resulted in an absorption coefficient of 100 cm^{-1}. The absorption of the dye increased linearly with concentration. An absorption of 100 cm^{-1} was chosen to match clot absorption at 506 nm. The gel was drawn up into the silicone tubes and allowed to cure.

2.2. Laser system

A 1 μs pulsed dye laser (Palomar Medical Technologies) operating at a wavelength 506 nm or 577 nm was used for ablation. The repetition rate was variable between 1–10 Hz. The laser delivery device consisted of a 400 μm quartz fiber contained within a 1 mm flexible catheter. The tip of the fiber did not project out of the catheter and was maintained at 1 mm from the catheter tip. The pulse energy was 50 mJ pulses out of the tip of the catheter.

2.3. Ablation

Light was delivered through the catheter with its tip about 1 mm from the target surface for the non-contact experiments. Contact experiments had the catheter tip touching the target surface. A steady flow of water at 4 ml/min was established by a fluid injector and directed through the catheter to the target site. Pulse repetition rates of 1 Hz, 3 Hz, and 6 Hz were used. Thirty pulses were fired on each sample and the ablated material was collected by the flowing water in 1 cm cuvettes. The steady flow was continued after the last pulse until 4 ml of liquid was collected. The above procedure was repeated on control samples without light delivery to account for material removed by the flow of water alone. The ablated gel dissolved completely in the water, and the hemoglobin lysed from the removed clot in about 10 minutes.

The total mass removed was determined by measuring the absorption of the dominant chromophore in the ablated material.[9] The chromophores are hemoglobin for clot and the dye Direct Red for gel. A calibration curve was used to convert the measured absorbance to the total mass removed. The calibration curves for clot and gel were established by measuring the absorbances of known masses of the material in 4 ml water. The curve was linear, and the slope of the line provided the necessary conversion factor.

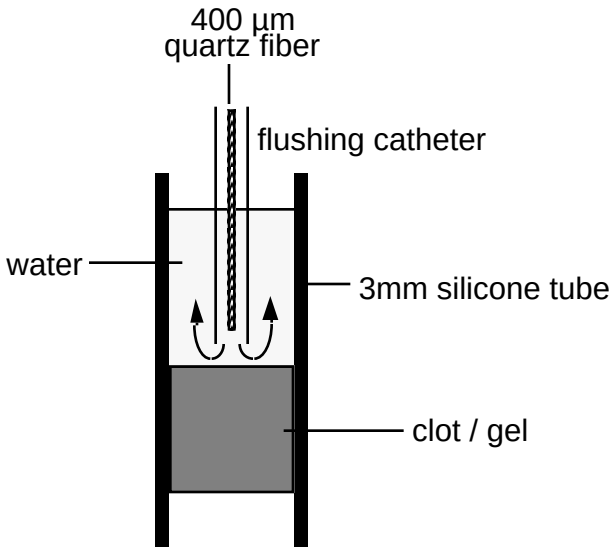

Figure 1. Porcine clot or gelatin is confined in a 3 mm diameter tube. Laser energy is delivered in 1 μs pulses via a flushing catheter to the target surface in either contact or non-contact mode. Water is flushed at 4 ml/min around the target site. The ablated material is collected in 4 ml water.

2.4. Visualization

Flash photography was used to visualize the ablation phenomenon. Images were captured at 20 μs or 100 μs after the laser pulse using a CCD camera and recorded on video. The time of image capture was controlled by a delay generator and a microsecond strobe. The trigger signal was provided by a photodiode detecting the laser pulse (Figure 2).

The CCD camera (CV-250, Motion Analysis, Eugene, OR) captures a frame once it gets a trigger from the delay generator. It then sends that frame to an image capture card on a computer or to the video tape recorder. The camera has a frame memory option where it stores the captured frame in a buffer and keeps sending the same frame to the frame grabber card or video recorder until it gets another trigger and captures another frame. An individual frame can therefore be recorded for several seconds on tape. This option is very useful for capturing the microsecond bubble action on video tape. The capture sequence is illustrated in figure (3).

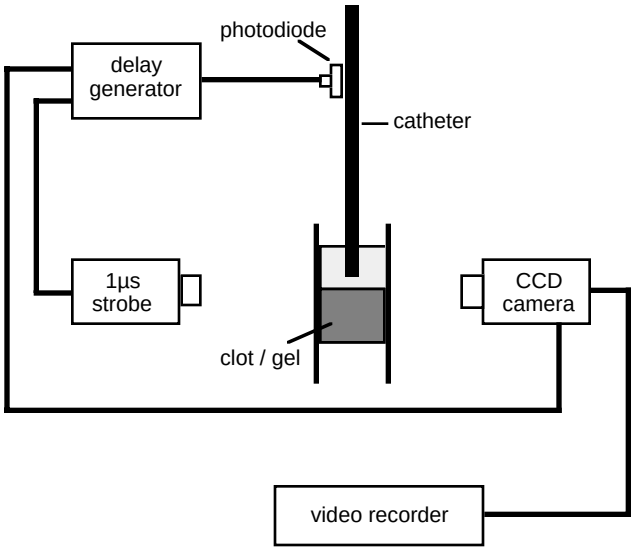

Figure 2. Flash photography: A 400 μm fiber contained in a flushing catheter was used to deliver 50 mJ. The photodiode detects the laser light and provides the trigger for the timing of image capture. The CCD camera captures a frame and sends it to the video recorder when it gets a trigger.

3. RESULTS

3.1. Ablation of gelatin

Ablation of gelatin was characterized by a snapping sound and visible removal of material. The gel was removed in small chunks which quickly dissolved in the water. All the ablated material was collected by the flowing water. There were no significant differences in the total mass ablated at pulse repetition rates of 1 Hz, 3 Hz, and 6 Hz (Figure 4).

Visualization of the ablation process showed clear bubble formation for the first 4–5 pulses. The images were captured 100 μs after the laser pulse. Figure (3) shows bubble formation at 100 μs in gel for a repetition rate of 1 Hz. The formation of a crater could be observed. The size of the bubble at 100 μs decreased with each pulse until no bubbles could be seen. However, a deepening crater was evident implying that ablation was still taking place. There were no significant differences in bubble action at 100 μs at the three repetition rates.

The gel was ablated with the catheter tip in contact with and 1 mm from the gel surface. The bubble action and total mass removed were similar for both cases. No pistoning of the catheter tip was observed (Figure 3).

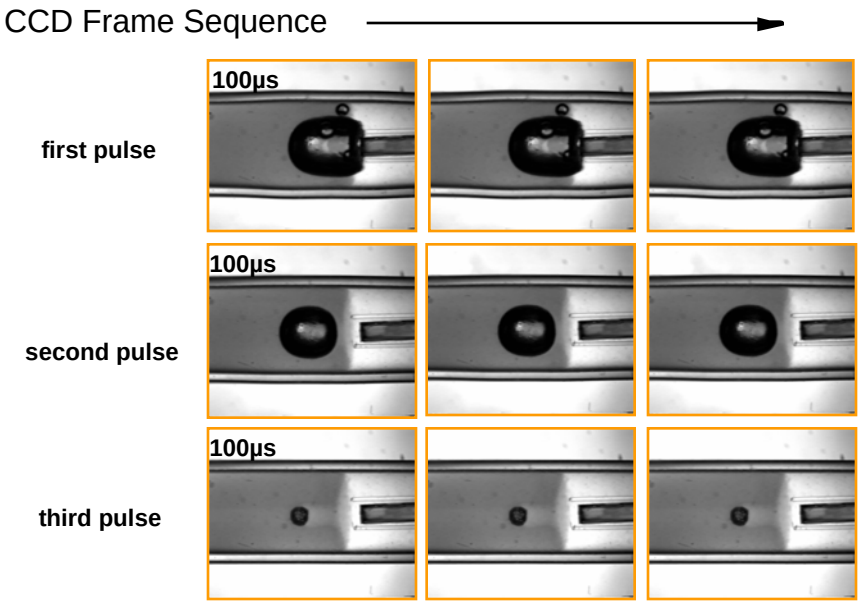

Figure 3. Ablation of gelatin at 1 Hz. The catheter tip is in contact with the gel surface. Images are captured 100 μs after the laser pulse. The bubble size becomes smaller with successive laser pulses. Crater formation can be observed. No pistoning of the catheter tip is observed due to contact. This figure also shows the frame sequence of the CCD camera. The camera captures a frame and stores it in memory. It keeps feeding that frame to the video recorder until it gets another trigger and captures a fresh frame.

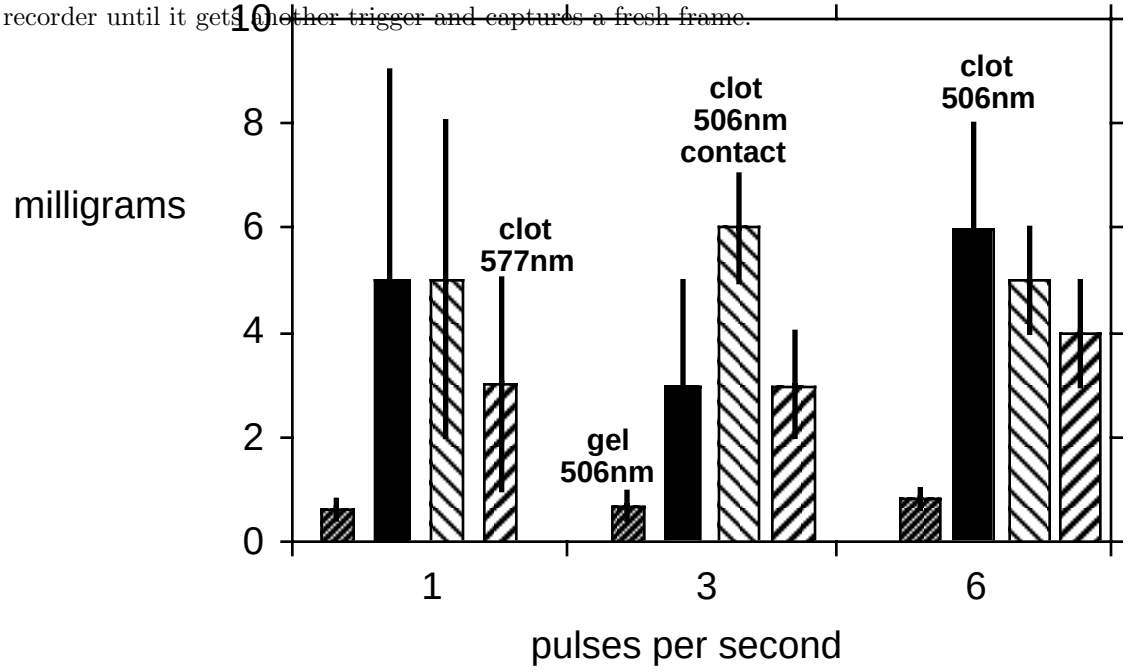

Figure 4. Ablation of clot and gel. More clot is removed than gel per pulse. Repetition rate has little effect on the total mass removed. The were no significant differences in ablation masses of clot between 506 nm ($\mu_a \approx 100\,\mathrm{cm}^{-1}$) and 577 nm ($\mu_a \approx 300\,\mathrm{cm}^{-1}$). Ablation was not affected by contact of the catheter with the clot.

3.2. Ablation of porcine clot

Ablation of clot was also characterized by a snapping sound and visible removal of clot. The ablated clot was removed in sizeable chunks of about 100–200 μm. The mass of clot removed was significantly higher than that of gel. The error in estimating the total mass was also higher. The three pulse repetition rates were equally efficient in ablating clot (Figure 4).

Ablation pictures were taken at 20 μs after the laser pulse (Figure 5). Bubbles were formed with each laser pulse delivered. Crater formation was not as clearly evident as with gel because the strobe light did not transilluminate the clot very well. However, a crater could be seen in the clot surface with a microscope. Ablation at higher pulse repetition rates seemed to be slightly more violent. However, mass removal measurements indicate equivalent ablation efficiencies.

The clot was ablated in contact and non-contact modes. Bubble behavior and mass removal were similar for both cases. No pistoning was seen with the catheter tip in contact with the clot surface.

Two wavelengths were used to ablate clot: 506 nm and 577 nm. The absorption of clot at these wavelengths is 100 cm^{-1} and 300 cm^{-1} respectively. The mass removal at both wavelengths was roughly equal indicating that the ablation efficiency is independent of absorption. Bubble action was also similar.

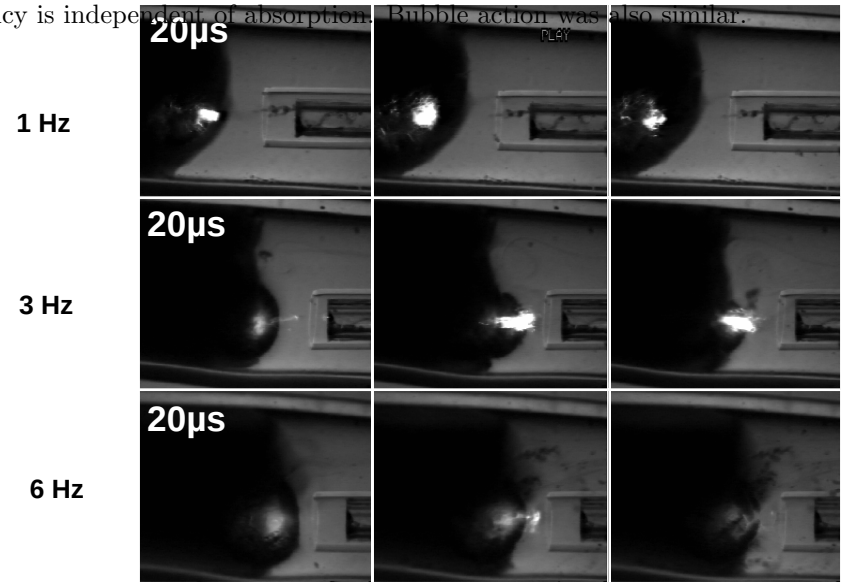

Figure 5. Clot ablation at 1 Hz, 3 Hz, and 6 Hz. Wavelength was 506 nm. The white light is the back-illuminating strobe light being transmitted through the bubble. Bubble action and ablation seems more violent at higher repetition rates, but the total mass removed was similar. Bubble action was similar at 577 nm.

4. DISCUSSION

This study was carried out to visualize the process of clot ablation and to investigate the effect of pulse repetition rate on the total mass removed. The clot was ablated with the catheter in contact with the clot surface to investigate a possible pistoning effect of the catheter tip. Pulse repetition rates of 1 Hz, 3 Hz, and 6 Hz were used to ablate porcine clot and gelatin (clot model) in both contact and non-contact modes. Images of bubble formation during the ablation process were captured on video tape with flash photography. The total mass removed was determined by quantifying the absorption signature of the ablated material. Wavelengths of 506 nm and 577 nm were used to study the effects of wavelength and absorption on the bubble formation and mass removed.

Significantly more clot than gel was removed with the thirty pulses delivered in both cases (figure 4). The higher ablation efficiency with clot is also evident in the flash photography experiment that was carried out at the same time as the mass removal experiments. Bubbles of almost equal size were created with every pulse while ablating clot. On the other hand, the bubbles in gel ablation got progressively smaller with successive pulses. After about

five pulses no bubbles could be seen at 100 μs although a deepening of the crater was observed. Acoustic reports were always heard. We hypothesize that the bubbles formed after the first five pulses were so small that they had already collapsed by the time the image was captured at 100 μs after the laser pulse. These smaller bubbles with shorter lifetimes had less ablative energies than bigger bubbles.

Ablation efficiencies were not affected by pulse repetition rate for both clot and gel (figure 4). This confirms the earlier postulated theory that efficiency is independent of repetition rate because the bubble lifetimes are much shorter than the time interval between pulses. Each laser pulse thus initiates an independent ablation event. The only influence a laser pulse has on the ablative efficiency of the next pulse is to change the target geometry due to removal of material and thus affecting bubble dynamics. Visualization of clot ablation at 1 Hz, 3 Hz, and 6 Hz seemed to indicate that more material is removed at higher repetition rates. This could be because the ablated material from the previous pulse had not yet been removed by the time the next pulse arrived. An important conclusion of this study is that thrombolysis could be performed at higher repetition rates without compromising efficiency. The total time for therapy would then be considerably shortened. However, the catheter used to deliver the laser pulses to the clot has an optical liquid core guide. Higher repetition rates may disrupt the flow of the liquid core due to more frequent bubble action.

Contact between the catheter tip and the target surface did not result in a pistoning effect of the tip. Such a pistoning effect effect had been suspected to be the cause of arterial dissections reported in some studies of laser thrombolysis and angioplasty. A source for such a force would have come from the bubble trying to expand against the catheter tip. However, as seen in this study, the vapor bubble expands readily into the clot and gel with the majority of the bubble volume being under the surface. Therefore not much force is exerted against the catheter and there is not much recoil. The pulse energy used here was 50 mJ which is close to that used in clinical settings. Higher energies will create more energetic bubbles that may exert force against the catheter tip causing recoil.

Finally, we compared clot ablation at 506 nm and 577 nm. Clot absorption varies from 100 cm^{-1} at 506 nm to 300 cm^{-1} at 577 nm. Our earlier parametric study with gelatin had predicted that ablation efficiency was largely independent of absorption. This was confirmed by the equivalent mass removals at 506 nm and 577 nm reported in this paper. Bubble action was also similar in the two cases. Laser thrombolysis can therefore be done at most visible wavelengths without compromising efficiency.

The main conclusions of this paper are: (*i*) bubble formation is similar on clot and gel. (*ii*) ablation efficiency is independent of pulse repetition rate between 1–6 Hz. (*iii*) contact does not result in pistoning with a pulse energy of 50 mJ. (*iv*) ablation efficiency and bubble formation in clot are similar at 506 nm and 577 nm. (*v*) clot ablation is about an order of magnitude more efficient than gel ablation.

5. ACKNOWLEDGEMENTS

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