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A study of blood clots using photoacoustic imaging during sonothrombolysis

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ABSTRACT

Formation of blood clots or thrombus in healthy blood vessels can lead to serious or even life-threatening complications. Sonothrombolysis is a promising tool for lysing the blood clots non-invasively using focused acoustic waves. Ultrasound (US) imaging is commonly used to detect the blood clots presents in veins. In this work, we explore the use of a combined ultrasound and photoacoustic (PA) imaging clinical system during sonothrombolysis. PA imaging is a hybrid and emerging imaging modality which has garnered the attention of the biomedical imaging community in recent years. While US imaging has been used to visualize the blood clot, PA imaging enables the study of composition of the blood clot due to its optical absorption. Blood clots may be red, white or mixed due to the higher count of red blood cells (RBCs), white blood cells (WBCs) or it being a combination of RBCs and WBCs, respectively. Each clot type has a different photoacoustic signal. In our work, blood clots rich in RBCs are taken in transparent polyurethane tubes for sonothrombolysis. Meanwhile, the ultrasound and photoacoustic signal-to-noise ratios (SNR) are measured at fixed time intervals to evaluate the size and optical properties of the clot. Two cases were taken: blood clot + DI water and blood clot + blood and their US and PA SNR values were compared after 30 mins of sonothrombolysis treatment. The PA signal of the blood clot obtained after performing sonothrombolysis can be used to determine its final composition which may, in turn, help in the administration of clot-dissolving drugs.

Keywords: blood clot, sonothrombolysis, ultrasound imaging, photoacoustic imaging, signal-to-noise (SNR) ratios, microbubbles.

1. INTRODUCTION

The blockage of blood vessels by blood clots or thrombi can lead to many life-threatening diseases[1] including ischemic stroke, myocardial infarction, pulmonary embolism (PE), deep vein thrombosis (DVT) and so on. The procedure to break the thrombus using focused ultrasound waves generated by acoustic transducers is called sonothrombolysis.[2] It has been reported that the thrombolysis efficiency is much higher if sonothrombolysis is accompanied by clot dissolving drugs such as tissue plasminogen activator (tPA),[3, 4] heparin,[5, 6] etc. Based on histopathological evaluation, it has been observed that the composition[7] of the thrombus is diverse. The composition[8] depends on the volume fractions and distribution of red blood cells (RBCs), white blood cells (WBCs), fibrin, platelets, etc. The effectiveness of clot dissolving drugs like tPA[3, 4] depends on the composition of the blood clot. Blood clots with higher fibrin content require a higher dosage of tPA compared to those which are rich with RBCs and WBCs. So, it will be beneficial to incorporate active diagnostic monitoring of the blood clot during sonothrombolysis. Currently, direct thrombus imaging is done using techniques such as compression ultrasound (US) for DVT[9] whereas Computed Tomography Pulmonary Angiography (CTPA)[10], ventilation/perfusion (V/Q)[11] and Magnetic Resonance Imaging (MRI)[12] scans have been used for PE. There are several drawbacks[13] to these imaging modalities. For instance, an US sonographer cannot determine the age and composition of the thrombus accurately.[14] With CTPA, there is a risk of contrast-induced nephropathy.[13] Moreover, CTPA and V/Q uses ionizing radiations.[15] MRI uses a strong magnetic field and cannot be used on patients with metal implants or pace-makers.[16]

Photoacoustic imaging (PAI)[17-20][21-23] uses non-ionizing radiation and is an emerging imaging modality. It offers unique advantages such as high spatial and temporal resolution, higher depth penetration, is speckle free and can provide both structural and functional information using various wavelengths. It utilizes a short-pulsed laser[24] on targeted biological tissues and detects the thermoelastic expansion using ultrasound transducers to identify a range of endogenous

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and exogenous molecules[25-30] with high sensitivity. Using combined ultrasound and photoacoustic (PA) imaging, Karpouk et al.,[31] reported that the amplitude of the photoacoustic signal is higher for an acute (fresh) clot compared to a chronic (long developing) clot at 532 nm. This is because the absorbance of oxygenated haemoglobin is higher than deoxygenated haemoglobin at this wavelength. In addition, the amplitude of the photoacoustic signal is directly proportional to the concentration of the RBCs[31] in the sample volume. PAI has also been demonstrated for label-free embolus detection[32] and for *in vivo* flow cytometry[33] of RBC and WBC rich blood clots using positive and negative contrast in the PA signal, respectively. Blood clots need to be observed in real time during sonothrombolysis as well to gain new insights into its mechanisms. Therefore, a dual modal ultrasound and photoacoustic imaging has been used in this work to actively monitor the blood clot. Combined US and PA imaging[34-37] provides sufficient spatial resolution and contrast with high sensitivity and specificity in real time. While ultrasound can provide structural information such as the size and density of the clot, photoacoustic imaging can also be used to analyse other important functional parameters such as change in the RBC and WBC concentration, haemoglobin concentration, haemoglobin oxygen saturation, etc.

2. METHODS

2.1 FORMATION OF BLOOD CLOT AND SONOTHROMBOLYSIS

For the formation of blood clots, porcine blood was collected from an abattoir (Primary Industries Pte. Ltd., Singapore) in 200 ml Schott glass bottles (Sigma, Singapore) and allowed to coagulate. First, experiments were performed using a blood clot + DI water in the tube. The clot was inserted in a polyurethane tube of outer diameter 4 mm and thickness 0.75 mm. Thereafter, DI water was injected into the tube. Once injected, the DI water was pushed and withdrawn repeatedly until the blood clot is surrounded by the DI water in the tube. Following this, experiment was performed using blood clot + blood in the tube. For this, porcine blood was collected from the same abattoir and was mixed with heparin with a concentration 30 units/ml to prevent it from coagulating during transportation. Prior to experiments, protamine sulfate (1 mg/100 units of heparin) was added to heparinized blood for reversing the effects of heparin. Thereafter, a blood clot was again inserted into a polyurethane tube following which the porcine blood containing heparin and protamine sulfate was injected into it.

Fig. 1 (a) Schematic illustration (top view) of the arrangement of the sonothrombolysis and imaging ultrasound transducers (UST) on the blood clot. The blood clot is taken in a polyurethane tube which itself is immersed in a water tank. The green region is used to illustrate the laser at 532 nm (b and c) Experimental images obtained by the imaging UST of the Alpinion E-Cube system when the sonothrombolysis UST was off (b) initially and later it was (c) turned on.

For sonothrombolysis, ultrasound waves were directed at the blood clot with a focused single element immersion transducer (0.5 MHz, V318-SU-F0.93IN-PTF, Olympus). A 10% duty cycle and an input voltage of 1 V_{p2p} was delivered by a function generator (AFG3101, Tektronix Inc.). The function generator was connected to a radio-frequency (rf)

amplifier (AG 1021, T&C Power Conversion, Inc., USA) which was operated at a gain of 55dB and 330 W (maximum amplification).

2.2 PHOTOACOUSTIC AND ULTRASOUND IMAGING OF THE BLOOD CLOT

Photoacoustic imaging of the blood clots was done with a clinical ultrasound-photoacoustic imaging system. A clinical research ultrasound system (ECUBE 12R, Alpinion, South Korea) was used. To use the system in the PA mode, laser excitation needs to be provided with a trigger,[37-39] which synchronizes the excitation of the laser and the signal acquisition by the ultrasound transducer. The blood clot containing tube was placed under water in a tank and a linear array ultrasound transducer L3-12 (128 element linear array with centre frequency of 8.5 MHz, 95% fractional bandwidth) was placed over the tube for sending and receiving the signals. A Q-switched 532-nm Nd: YAG laser, was used as an excitation source for PA spectrum measurements. The laser generates 5-ns duration pulses at 10-Hz repetition rate.

As mentioned previously, a spherically focused single element immersion transducer was used for sonothrombolysis in this work. The vibration of water surrounding the tube containing the blood clot due to the acoustic waves generated by the single element immersion transducer and it results in an echo which is received by the imaging transducer and are clearly visible on the clinical research ultrasound system in the B-mode. This has been shown in Figs. 1(b) and (c).

3. RESULTS AND DISCUSSIONS

To investigate the change in composition of the blood clot during sonothrombolysis, experiments were performed using blood clot + DI water and using blood clot + blood in the polyurethane tube. The arrangement of the sonothrombolysis

Fig. 1 (a-d) Raw experimental images on the ECUBE system obtained for ultrasound (US) and photoacoustic (PA) imaging where (a), (c) shows the blood clot in a tube filled with DI water at time, $t = 0$ mins; (b), (d) shows the diminished blood clot in a tube filled with DI water at time, $t = 30$ mins. The signal-to-noise ratios (SNR) are shown for each figure. (e) Schematic illustration of the red blood cells (RBCs) expelled from the blood clot during sonothrombolysis which results in a drop in PA SNR values.

and imaging transducers is shown in Fig. 1 (a) from the top view. The sonothrombolysis work presented in this report is performed without any thrombolytic agents. The experimental images in the ultrasound mode and photoacoustic mode

are shown in Figs. 2 and 3. The blood clots have been highlighted. The signal-to-noise (SNR) is given in decibels (dB) by $SNR = 20 \times \log_{10}(V/n)$, where V is the PA signal amplitude and n is the mean of the background noise. The blood clot is taken in DI water in Fig. 2 and in blood in Fig. 3. DI water is used to simulate the presence of saline. It is to be noted that the blood clots used in this work are not controlled ones and hence, the initial weight of both will vary in Figs. 2 and 3.

For blood clot + DI water experiment [Figs. 2(a, b)] in the tube, at time, $t = 0$ mins; US SNR and PA SNR of the blood clot in blood was 49.4 dB and 40.4 dB. Thereafter, US and PA images were collected after mediating sonothrombolysis for 30 minutes continuously. The blood clot appeared diminished and SNR values of US and PA reduced to 42.9 dB and 15.8 dB, respectively. This has been shown in Figs. 2(c, d) and implies, the US and PA SNR values reduced by 13.2% and 61%, respectively. Next, experiments were performed with blood clot + blood (Fig. 3) in the tube. At time, $t = 0$ mins [Fig. 3 (a, b)]; the US and PA SNR values were 18.3 dB and 47 dB. After sonothrombolysis treatment of 30 mins, the US and PA SNR values were reduced to 17 dB and 39.4 dB, respectively as shown in Figs. 3(c, d). In this case, the US and PA SNR values diminished by 7.1 % and 16.2 %, respectively. Since the blood clots used in the two cases: blood clot + DI water and blood clot + blood, are different; the SNR values fell by different percentages. However, there is a significant change in the PA SNR values between the two cases. This occurred due to the greater diffusion of RBCs from the blood clot to DI water compared to blood. It has already been reported that the concentration of RBCs determines the PA signal amplitude.[31] The importance of this work is that in a real scenario, where the clinicians observe a significant drop in the PA signal amplitude of the blood clot during sonothrombolysis, but the US SNR does not change much, it will provide a feedback to the doctor that the clot is rich in fibrin and platelets. In such a case, a higher dosage of clot dissolving drugs such as tPA[4] needs to be administered.

Fig. 2 Raw experimental images on the ECUBE system obtained for ultrasound (US) and photoacoustic (PA) imaging where (a), (b) shows the blood clot in a tube filled with blood at time, $t = 0$ min; (c), (d) shows the blood clot in a tube filled with blood at time, $t = 30$ mins. The signal-to-noise ratios (SNR) are shown for each figure.

4. CONCLUSION

A combined US and PA imaging set up was used to determine the relative changes in the composition of porcine blood clots before and after sonothrombolysis for two cases: blood clot + DI water and blood clot + blood. As the blood clots used in this work were not controlled, the US and PA SNR values were also different before and after sonothrombolysis for two above mentioned cases. However, there is a significant reduction in the PA SNR value for the first case due to the diffusion of RBCs from the blood clot to the surrounding DI water. This was achieved without the use of any

exogenous contrast agent due to the rich optical contrast of the blood clot. Hence, for a blood clot which has a rapidly decreasing PA SNR value compared to the US SNR value, it implies that the clot is rich in fibrin and platelets in comparison to its surroundings. An insight into the clot composition during sonothrombolysis can be used to facilitate treatment approaches which can expedite the recovery of the patient.

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6. REFERENCES

- [1] S. Z. Goldhaber, and H. Bounameaux, "Pulmonary embolism and deep vein thrombosis," *Lancet*, 379(9828), 1835-46 (2012).
- [2] S. T. Roos, L. J. M. Juffermans, J. Slikkerveer *et al.*, "Sonothrombolysis in acute stroke and myocardial infarction: A systematic review," *IJC Heart & Vessels*, 4, 1-6 (2014).
- [3] K. Kirchhof, M. Sikinger, T. Welzel *et al.*, "[Does the result of thrombolysis with recombinant tissue-type plasminogen activator (rt-PA) in rabbits depend on the erythrocyte- and fibrin-content of a thrombus?]," *Rofo*, 176(1), 98-105 (2004).
- [4] I. K. Jang, H. K. Gold, A. A. Ziskind *et al.*, "Differential sensitivity of erythrocyte-rich and platelet-rich arterial thrombi to lysis with recombinant tissue-type plasminogen activator. A possible explanation for resistance to coronary thrombolysis," *Circulation*, 79(4), 920-8 (1989).
- [5] S. Z. Goldhaber, and V. F. Tapson, "A prospective registry of 5,451 patients with ultrasound-confirmed deep vein thrombosis," *The American Journal of Cardiology*, 93(2), 259-262 (2004).
- [6] S. Atar, Y. Neuman, T. Miyamoto *et al.*, "Synergism of aspirin and heparin with a low-frequency non-invasive ultrasound system for augmentation of in-vitro clot lysis," *J Thromb Thrombolysis*, 15(3), 165-9 (2003).
- [7] P. Singh, R. Kaur, and A. Kaur, "Clot composition and treatment approach to acute ischemic stroke: The road so far," *Annals of Indian Academy of Neurology*, 16(4), 494-497 (2013).
- [8] J. Minnerup, and C. Kleinschnitz, "Visualization of clot composition in ischemic stroke: Do we get what we see?," *Stroke*, 42(5), 1193-1194 (2011).
- [9] S. M. Bates, R. Jaeschke, S. M. Stevens *et al.*, "Diagnosis of DVT: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines," *Chest*, 141(2 Suppl), e351S-e418S (2012).
- [10] C. R. Weiss, J. C. Scatarige, G. B. Diette *et al.*, "CT Pulmonary Angiography is the First-Line Imaging Test for Acute Pulmonary Embolism: A Survey of US Clinicians," *Academic Radiology*, 13(4), 434-446 (2006).
- [11] H. Gutte, J. Mortensen, C. V. Jensen *et al.*, "Comparison of V/Q SPECT and planar V/Q lung scintigraphy in diagnosing acute pulmonary embolism," *Nuclear Medicine Communications*, 31(1), 82-86 (2010).
- [12] E. J. R. van Beek, J. M. Wild, C. Fink *et al.*, "MRI for the diagnosis of pulmonary embolism," *Journal of Magnetic Resonance Imaging*, 18(6), 627-640 (2003).
- [13] M. V. Huisman, and F. A. Klok, "Current challenges in diagnostic imaging of venous thromboembolism," *Blood*, 126(21), 2376-82 (2015).
- [14] B. Dharmarajah, V. Sounderajah, S. P. Rowland *et al.*, "Aging techniques for deep vein thrombosis: a systematic review," *Phlebology*, 30(2), 77-84 (2015).
- [15] A. M. Groves, S. J. Yates, T. Win *et al.*, "CT Pulmonary Angiography versus Ventilation-Perfusion Scintigraphy in Pregnancy: Implications from a UK Survey of Doctors' Knowledge of Radiation Exposure," *Radiology*, 240(3), 765-770 (2006).
- [16] E. T. Martin, and D. A. Sandler, "MRI in patients with cardiac devices," *Current Cardiology Reports*, 9(1), 63-71 (2007).
- [17] J. Yao, and L. V. Wang, "Recent progress in photoacoustic molecular imaging," *Current Opinion in Chemical Biology*, 45, 104-112 (2018).
- [18] P. K. Upputuri, and M. Pramanik, "Fast photoacoustic imaging systems using pulsed laser diodes: a review," *Biomedical Engineering Letters*, 8(2), 167-181 (2018).
- [19] P. K. Upputuri, and M. Pramanik, "Recent advances toward preclinical and clinical translation of photoacoustic tomography: a review," *Journal of Biomedical Optics*, 22(4), 041006 (2017).

- [20] L. V. Wang, and S. Hu, "Photoacoustic Tomography: In Vivo Imaging from Organelles to Organs," *Science*, 335(6075), 1458-1462 (2012).
- [21] P. K. Upputuri, and M. Pramanik, "Performance characterization of low-cost, high-speed, portable pulsed laser diode photoacoustic tomography (PLD-PAT) system," *Biomedical Optics Express*, 6(10), 4118-29 (2015).
- [22] P. K. Upputuri, K. Sivasubramanian, C. S. K. Mark *et al.*, "Recent developments in vascular imaging techniques in tissue engineering and regenerative medicine," *BioMed Research International*, 2015, 9 (2015).
- [23] S. K. Kalva, P. K. Upputuri, and M. Pramanik, "High-speed, low-cost, pulsed-laser-diode-based second-generation desktop photoacoustic tomography system," *Optics Letters*, 44(1), 81-84 (2019).
- [24] L. V. Wang, and J. Yao, "A practical guide to photoacoustic tomography in the life sciences," *Nature Methods*, 13(8), 627-38 (2016).
- [25] K. Sivasubramanian, M. Mathiyazhakan, C. Wiraja *et al.*, "Near Infrared light-responsive liposomal contrast agent for photoacoustic imaging and drug release applications," *Journal of Biomedical Optics*, 22(4), 041007 (2017).
- [26] M. Mathiyazhakan, P. K. Upputuri, K. Sivasubramanian *et al.*, "In situ synthesis of gold nanostars within liposomes for controlled drug release and photoacoustic imaging," *Science China Materials*, 59(11), 892-900 (2016).
- [27] C. Wiraja, M. Mathiyazhakan, F. Movahedi *et al.*, "Near-infrared light-sensitive liposomes for enhanced plasmid DNA transfection," *Bioengineering & Translational Medicine*, 1(3), 357-364 (2016).
- [28] Y. Jiang, P. K. Upputuri, C. Xie *et al.*, "Broadband Absorbing Semiconducting Polymer Nanoparticles for Photoacoustic Imaging in Second Near-Infrared Window," *Nano Letters*, 17(8), 4964-4969 (2017).
- [29] Z. Wang, P. K. Upputuri, X. Zhen *et al.*, "pH-sensitive and biodegradable charge-transfer nanocomplex for second near-infrared photoacoustic tumor imaging," *Nano Research*, 12(1), 49-55 (2019).
- [30] J. Zhou, Y. Jiang, S. Hou *et al.*, "Compact Plasmonic Blackbody for Cancer Theranosis in Near-Infrared II Window," *ACS Nano*, 12(3), 2643-2651 (2018).
- [31] A. B. Karpouk, S. R. Aglyamov, S. Mallidi *et al.*, "Combined ultrasound and photoacoustic imaging to detect and stage deep vein thrombosis: phantom and ex vivo studies," *Journal of Biomedical Optics*, 13(5), 054061 (2008).
- [32] M. A. Juratli, Y. A. Menyaev, M. Sarimollaoglu *et al.*, "Real-Time Label-Free Embolus Detection Using In Vivo Photoacoustic Flow Cytometry," *PLOS ONE*, 11(5), e0156269 (2016).
- [33] E. I. Galanzha, M. Sarimollaoglu, D. A. Nedosekin *et al.*, "In vivo flow cytometry of circulating clots using negative photothermal and photoacoustic contrasts," *Cytometry*, 79(10), 814-824 (2011).
- [34] D. Das, K. Sivasubramanian, C. Yang *et al.*, "On-chip generation of microbubbles in photoacoustic contrast agents for dual modal ultrasound/photoacoustic in vivo animal imaging," *Scientific Reports*, 8(1), 6401 (2018).
- [35] K. Sivasubramanian, V. Periyasamy, R. A. Dienzo *et al.*, "Hand-held, clinical dual mode ultrasound-photoacoustic imaging of rat urinary bladder and its applications," *Journal of Biophotonics*, 11(5), e201700317 (2018).
- [36] K. Sivasubramanian, V. Periyasamy, and M. Pramanik, "Non-invasive sentinel lymph node mapping and needle guidance using clinical handheld photoacoustic imaging system in small animal," *Journal of Biophotonics*, 11(1), e201700061 (2018).
- [37] K. Sivasubramanian, and M. Pramanik, "High frame rate photoacoustic imaging at 7000 frames per second using clinical ultrasound system," *Biomedical Optics Express*, 7(2), 312-323 (2016).
- [38] K. Sivasubramanian, V. Periyasamy, K. K. Wen *et al.*, "Optimizing light delivery through fiber bundle in photoacoustic imaging with clinical ultrasound system: Monte Carlo simulation and experimental validation," *Journal of Biomedical Optics*, 22(4), 041008 (2017).
- [39] K. Sivasubramanian, V. Periyasamy, and M. Pramanik, "Hand-held clinical photoacoustic imaging system for real-time non-invasive small animal imaging," *Journal of Visualized Experiments*, 128, e56649 (2017).