Sonothrombolysis – Dissolving Thrombi by Interaction of the Drug and Ultrasound

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***Abstract*—Under the influence of pathological changes, the blood coagulates inside the blood vessel, creating a thrombus. The thrombus dissolution process is called thrombolysis. The aim of the study is to evaluate the thrombolysis process by the interaction of the thrombolitic drug and ultrasound. The clot dissolution process was analyzed in a specially designed, transparent for ultrasound parallel plate flow chamber. Inside, a freshly coagulated human blood sample was exposed to ultrasound. A liquid containing the tissue plasminogen activator drug in a concentration of 10 µg/ml passed around the sample. The liquid flow was forced by a peristaltic pump. The source of ultrasound was a 1 MHz flat ultrasonic transducer with a 25 mm diameter. The transducer radiated a 1000 periods burst, repeated every 2500 periods and space averaged time averaged intensities of 0.2-1.6 W/cm^2. The efficacy of thrombus dissolution was observed by means of a designed parallel plate flow chamber and the time of thrombus complete dissolution was measured. The best result for the 1 MHz frequency and space averaged time averaged intensity of 1.6 W/cm^2 was recorded, where the thrombus was dissolved within 5.5 minutes.**

Keywords—ultrasound, blood, thrombus, thrombolysis, parallel plate flow chamber,

I. Introduction

Diseases of the cardiovascular system are the leading cause of death both in Poland and in the world. Formation of blood clots within blood vessels with ruptured atherosclerotic plaque plays the major role in the pathogenesis of heart attacks and strokes. It is crucial to remove the thrombus related occlusion of the vessel as soon as possible. There are two methods: intervention through mechanical recanalization of the artery to the area of infarction using a leader with subsequent stent placement and dissolving a blood clot within a few hours after formation. The last procedure, known as thrombolytic therapy, is the administration of drugs such as a tissue plasminogen activator (tPA), causing fibrinolysis, leading to dissolution of the thrombus.

Alexandrov et al [1] have found that the process of thrombolysis can be accelerated by administration of a clot dissolving drug and simultaneous sonication of the occluded region with ultrasound. This speeds revascularization, reducing the time and lowering the dose of the drug therapy compared with standard procedure [2]. The effectiveness of the combined drug ultrasound method, called sonotrombolyis has been confirmed in the laboratory and clinical studies by many researchers. However, the origin of the phenomenon of ultrasound wave interaction and the thrombolytic drug remains unknown.

The aim of this study was to evaluate the process of thrombolysis by the interaction of the drug and the ultrasonic wave. The scope of work involved the study of the influence of frequency, sound pressure, intensity and power of ultrasonic wave on the process of dissolution of thrombus. The efficacy of the thrombolysis process was assessed on the basis of measurement of time for completely dissolved blood clots. The project was carried out jointly with the Military Medical Institute in Warsaw.

The parallel plate flow chamber is widely used to study the interaction between cells and a flowing liquid. For the in-vitro analysis of the blood clotting process, parallel plate flow chambers have been used repeatedly [3]. They are also used for fibrinolysis research [4]. Commercial parallel plate flow chambers are made of glass and metal which limits or makes impossible the use of an ultrasound wave in the sonothrombolysis process. The authors have designed and developed a transparent for ultrasound parallel plate flow chamber to analyze the thrombolysis process under the interaction of acoustic wave and the tissue plasminogen activator drug.

II. Experiment

A. Flow Chamber

The designed parallel plate flow chamber (Fig.1) consisted of four ultrasound transparent elements (Fig.2). The base has been made of rexolite (C-Lec Plastics, Inc., Philadelphia, USA) with an 8.5 mm thickness. Rexolite was selected due to its low ultrasound attenuation coefficient and low water absorption [5]. Two longitudinal holes 20 mm distant from which the liquid was flowing in and out were milled in the base. A 40 mm diameter recess holding the gasket and the top cover was made. The gasket determined the width and height of the flow channel. The 1 mm silicone gasket A2414 from Bioptechs Inc.,

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Butler, PA, USA, and a channel width of 11 mm was used. The cover was made of 1 mm thick polycarbonate. The cover and gasket were pressed by the upper ring fastened by six screws. The surfaces of rexolite and polycarbonate have been polished to allow undistorted observation of the clotted blood sample through a microscope or camera for macro photography.

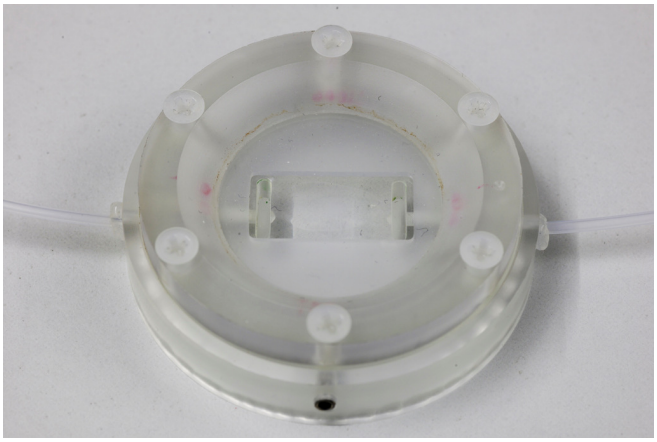

Fig. 1. Designed transparent for ultrasound parallel plate flow chamber.

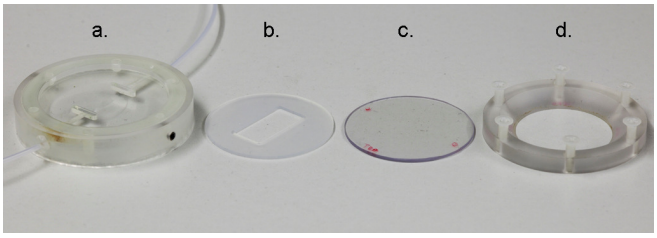

Fig. 2. Four elements of a parallel plate flow chamber: a. rexolite base, b. silicone gasket, c. polycarbonate cover, d. upper ring.

B. *Measurements*

The prepared parallel plate flow chamber was placed on the top of a multi-walled tank made of polycarbonate (Fig. 3). An ultrasonic transducer was bolted to one of the oblique walls, and a layer of silicone rubber (Silikony Polskie, Poland), serving as an ultrasonic absorber, was poured onto the opposite sloping wall. A flat LED illuminator, which was a light source, was placed under the horizontal bottom wall. The tank was filled with distilled and degassed water at a 37°C temperature and a 500 ml volume. A flat ultrasonic transducer with a diameter of 25 mm and a 1 MHz frequency was used (Pz28, Meggitt, Denmark). The transducer emitted an ultrasound wave at a 45° angle upwards and towards the chamber with a thrombus. Then the ultrasound wave was reflected from the upper surface of the chamber and returned to the ultrasonic absorber. Because the reflected wave propagated perpendicular to the incident wave, there was no interference between them and there was no standing wave phenomenon.

To verify the parallel plate flow chamber transparency for ultrasound, the transverse distribution of the acoustic pressure generated by the ultrasonic transducer was measured. The measurement was made at a distance of 104 mm from the transducer, at the border of the near field (a^2/λ). The transverse distribution (in the z axis) was measured in the range of ±12.5 mm. The pressure was measured with a 0.2 mm needle hydrophone (Precision Acoustics, UK) connected to a DSO9410A digital oscilloscope (Agilent, USA). The distribution of the acoustical field was tested: in water (a), and with the bottom plate of the rexolite chamber placed perpendicular to the ultrasonic beam (b), and at an angle of 45° to the beam, as in the measuring vessel (c).

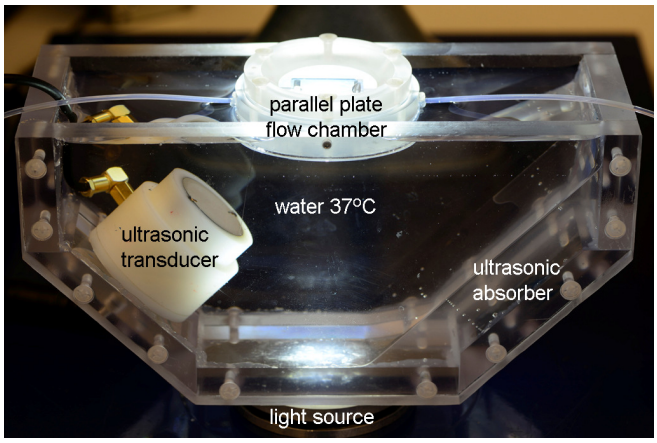

Fig. 3. Multi-walled tank used for the sonication of the thrombus inside the parallel plate flow chamber.

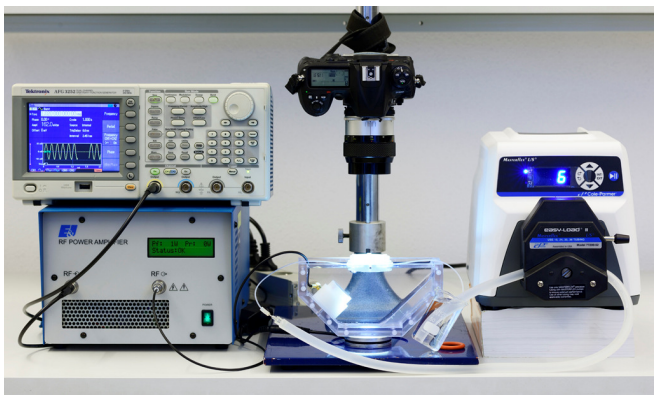

Fig. 4. Laboratory set-up used for the sonication of the thrombus (waveform generator and rf power amplifier on left, photographic camera and multi-walled tank in center and peristaltic pump on right).

The diagram of the measurement set-up is shown in Fig. 4. The ultrasonic transducer was excited by a 1000 periods sinusoidal burst with the 1 MHz frequency repeated every 2.5 ms. The duty factor was t/T = 0.4. The ultrasound frequency of 1 MHz was chosen based on the publication of other authors [6]. The waveform was generated by the AFG3225 function generator (Tektronix, USA) and amplified by the 325LA radio frequency amplifier (Electronics & Innovation, Ltd., Rochester, NY, USA). The ultrasonic wave was generated at intensities 0.2-1.6 W/cm^2. The ultrasound intensity was determined using an ultrasound power meter UPM-DT-1E (Ohmic Instruments, Easton, MD, USA). The flow of liquid inside the parallel plate flow chamber was forced by a Masterflex L/S peristaltic pump (Cole-Parmer, USA). The average flow rate was 18 ml/min at a pump speed of 6 rpm. The flowing liquid was a Dulbecco's Modified Eagle's Medium solution 51449C, with 4500 mg/L dextrose, with 110 mg/L sodium pyruvate, without L-glutamine, without phenol red (Sigma-Aldrich, Germany). The tissue plasminogen activator drug in a concentration of 10 µg/ml was added to the liquid (Actilyse, Boehringer Ingelheim, Ingelheim am Rhein, Germany).

With the consent of the local ethics committee, 4 ml of a human blood sample was collected. The blood in the clean test tubes was left to solidify for 60 min at 37°C. A thrombus with an average weight of 1.9 g was obtained, which was cut into slices with 8 mm diameter and 1 mm thickness. The prepared thrombus samples were placed in the parallel plate flow chamber and sonicated for 10 min or until the thrombus was completely dissolved. Thrombi during dissolution was photographed every 30 seconds. Then, the photos were analyzed by measuring the thrombus area. Assuming a constant thickness of the sample, the change in thrombus volume was determined.

III. Results

The measured transverse distribution of the acoustic field is shown in Fig.5.

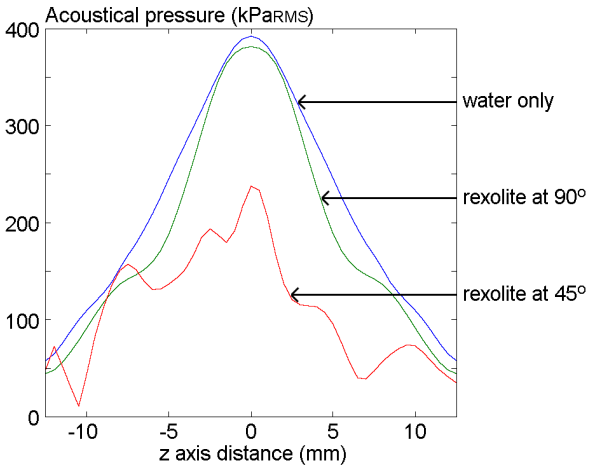

Fig. 5. Measured transverse distribution of the acoustic field for the 25 mm diameter, 1 MHz ultrasonic transducer on the near field boundary, at a distance of a^2/λ = 104 mm. Measurements were made in water (a), and with the rexolite plate placed perpendicular to the ultrasonic beam (b), and at an angle of 45° to the beam (c).

The maximum root mean square pressure value, measured on the transducer axis, was 392 kPa_{RMS}. The 8.5 mm thick rexolite plate reduced the axis pressure to 382 kPa_{RMS} (by 3%). Narrowing the beam and reducing the pressure caused a total reduction of the sound power by 24%. The power was calculated as the sum of the acoustic intensities on the surface equal to the transducer surface. Placing the polystyrene plate at an angle of 45°, just like in a measuring tank, resulted in a reduction of the pressure on the transducer's axis to 238 kPa_{RMS}. Uneven transverse pressure distribution and the supposed lack of axial symmetry made it impossible to calculate the sound power. The estimated loss of sound power was 66-75%.

A closed (assembled) parallel plate flow chamber before sonication flooded with Dulbecco's Modified Eagle's Medium liquid and with the thrombus inside is shown in Fig.6.

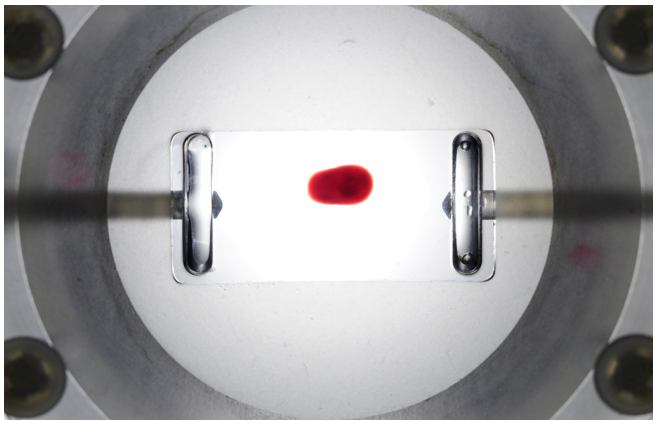

Fig. 6. Parallel plate flow chamber flooded with liquid and with the thrombus inside.

The next picture shows the sequence of thrombus pictures during the sonication process (Fig. 7). The pictures were taken every 2 minutes. Sonication was carried out at an ultrasonic intensity of 0.8 W/cm^2 in 10 min.

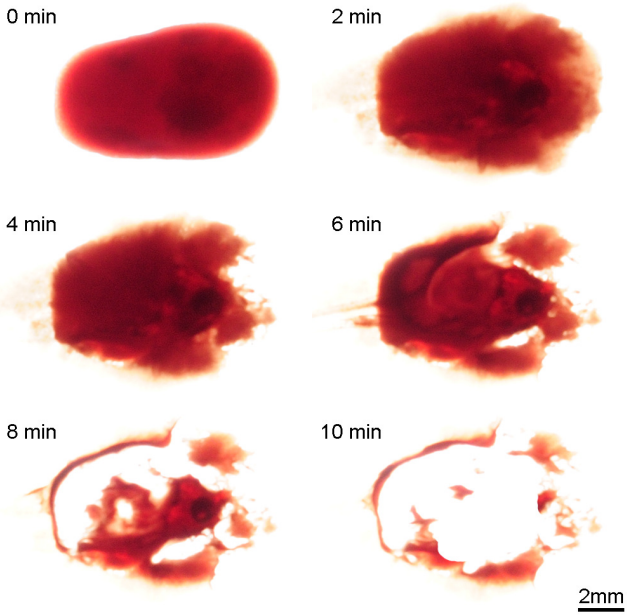

Fig. 7. Sequence of the thrombus images during the thrombus dissolution process at ultrasound intensity 0.8 W/cm^2 during 10 min sonication.

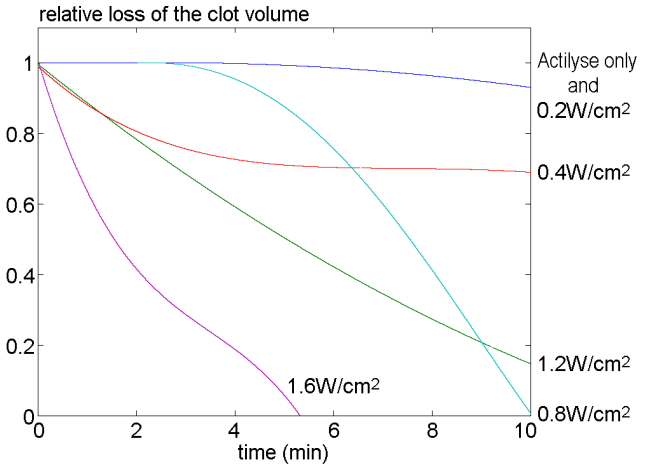

Fig. 8. Relative loss of thrombus volume for different values of ultrasound intensity in the range 0.2-1.6 W/cm^2 and 0 W/cm^2, in the absence of sonication (Actilyse only).

Finally, the relative loss of thrombus volume is shown in Fig. 8 and Table 1. Graphs for the space averaged, time averaged ultrasound intensities I_{SATA} in the range of 0.2-1.6 W/cm^2 are presented. Graphs for 0 W/cm^2 (Actilyse only) and 0.2 W/cm^2 are identical.

TABLE I. RELATIVE LOSS OF THROMBUS VOLUME

ultrasound intensity	relative loss of trombus volume	
	loss after 10 min sonication	*time for 100% loss*
0	7 %	-
0.2 W/cm^2	7 %	-
0.4 W/cm^2	31 %	-
0.8 W/cm^2	100 %	10 min
1.2 W/cm^2	85%	> 10 min
1.6 W/cm^2	100 %	5.5 min

For I_{SATA} = 0.2 W/cm^2, no effect of the ultrasound on the thrombus was noticed. After 10 min, the thrombus volume decreased to 93%, in the same way as in the absence of ultrasound. The threshold value was 0.4W/cm^2, where the thrombus volume decreased to 69% in 5 min. Over the next 5 min the volume of thrombus remained unchanged. For 0.8 W/cm^2 and 1.2 W/cm^2 intensities, the thrombus volume was reduced to 0% and 15%, respectively, within 10 min exposure. At the highest ultrasound intensity of the 1.6 W/cm^2, the thrombus was completely dissolved in 5.5 min.

IV. CONCLUSIONS

The transparent for ultrasound parallel plate flow chamber allowed us to study the interaction of the tissue plasminogen activator Actilyse drug and the ultrasound wave into the thrombus.

In the designed parallel plate flow chamber the influence of standing wave was eliminated, because the wave incident on the thrombus was perpendicular to the wave reflected at the polycarbonate (cover plate) and air boundary. The use of rexolite reduced the losses to a minimum estimated at 66-75% compared to the free space. The expected threshold intensity of the ultrasound necessary to dissolve the thrombus should be higher than in the presence of standing wave [7]. However the I_{SATA} ultrasound intensity threshold value was 0.4 W/cm^2, similar 0.32 W/cm^2 threshold obtained in another in-vitro experiment involving the interaction of C6 glioma tumor cells and ultrasound [7]. The small distance (1 mm) between the thrombus and the surface reflecting the ultrasound wave could be the cause of the improvement of the thrombus dissolution efficiency.

At ultrasound intensities of 0.8 W/cm^2 to 1.6 W/cm^2, complete thrombus dissolution was achieved, which confirmed the effectiveness of the new method of interaction of the ultrasound and thrombolytic drug.

The construction of the measurement setup enabled the installation of ultrasonic transducers at different frequencies and finding the optimal ultrasound frequency for the sonotrombolysis process. In the future, special attention will be paid to low ultrasound frequencies, starting at 40 kHz [8].

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