Project Acronym:

SOUNDPET(INTEGRATED/0918/0008)

MRI-guided Focused ultraSOUND system for cancer in PETs (dogs and cats).

Deliverable number: 6.5

Title: Evaluation of the system in performing ablation in rabbits.

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Date: 15/5/2023

Ευρωπαϊκή Ένωση
Ευρωπαϊκά Διαρθρωτικά και Επενδυτικά Ταμεία

Κυπριακή Δημοκρατία

Διαρθρωτικά Ταμεία της Ευρωπαϊκής Ένωσης στην Κύπρο

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Executive Summary

In this deliverable, the evaluation of two versions of the SOUNDPET robotic device in healthy rabbits is described. Both versions of the robotic device were used to evaluate the *in vivo* efficacy of each system by ablating healthy rabbit thigh tissue in a laboratory setting. The two robotic systems employed 3 linear axes (X, Y, Z) and one angular axis (Θ). Due to limitations in the target size (rabbit thigh), motion in the Z and Θ axes was not required. The first version of the robotic device included small piezoelectric motors and a low-frequency transducer (1.1 MHz), while the second version included large piezoelectric motors and a higher frequency transducer (2.6 MHz).

Forty (40) experimental rabbits (72 thighs) were included in the evaluation study of the *in vivo* efficacy of both versions of the robotic device. Specifically, 20 animals were used for assessment of the first version of the device, and 20 for the second version. During the experiments, the appropriate procedure for rabbit preparation was followed, thus ensuring no indication of animal suffering. All animals remained in deep general anesthesia until euthanasia.

Both versions were tested by appropriate choice of the ultrasonic parameters and the robotic motion grid patterns using the developed electronic software. In this regard, during the experiments, different focal depth, acoustic power, sonication time, grid pattern, and step movement between successive sonications were used. By adjusting the aforesaid parameters, both discrete and overlapping lesions were produced with variable lesion diameter and length, thus demonstrating the efficacy of the system in creating reproducible and controllable lesions. In addition, ablation was limited within the targeted tissue without damaging other areas. The necessary anesthesia monitoring ensures that the ablation procedure possesses no threat to animal welfare and wellbeing. Additionally, no adverse effects related to system safety were observed during the experiments.

Introduction

After the development of the first version of the robotic device (Deliverable 3.1) and evaluation of both the accuracy of motion (Deliverable 6.2) and thermal heating of the transducer (Deliverable 6.3), experiments were conducted on 20 rabbits in the laboratory setting. The experiments were performed following the requirements set by the Animal Welfare Committee of the SOUNDPET project application, thus ensuring maximal animal wellbeing. All rabbits were sourced from an accredited supplier (S. Ioannou, Limassol, Cyprus) who possessed the required license (CYCU5.05). The SOUNDPET project received the license (number: CY/EXP/PR.L01/2020) required for execution of the rabbit experiments from the Veterinary Services (Ministry of Agriculture, Rural Development and Environment) of the Republic of Cyprus. The experiments were supervised by a qualified veterinarian (Kyriakos Spanoudes) who was approved by the Animal Welfare Committee to participate in the SOUNDPET experiments executed on rabbit models. In each experiment, an anesthesia record containing the necessary information, anesthesia timeline and vital signs monitoring was filled by the qualified veterinarian, thus providing records regarding the experimental procedure and the welfare of the rabbit subject. The template of the anesthesia record is shown in Table 1.

Table 1: Sample of the anesthesia record that was filled out for each rabbit experiment.

<table>
<tr><td colspan="3">Animal ID</td><td colspan="3">SOUNDPET_2020_</td></tr>
<tr><td colspan="3">Species</td><td colspan="3">Rabbit</td></tr>
<tr><td colspan="3">Sex</td><td colspan="3">M/F</td></tr>
<tr><td colspan="3">Age</td><td colspan="3">Adult</td></tr>
<tr><td colspan="3">Weight (Kg)</td><td colspan="3"></td></tr>
<tr><td colspan="3">Supplier</td><td colspan="3">S. Ioannou Farm (CYCU5.05)</td></tr>
<tr><td colspan="3">Grant</td><td colspan="3">SOUNDPET (INTEGRATED/0918/0008)</td></tr>
<tr><td colspan="3">Study</td><td colspan="3">CY/EXP/PR.L01/2020</td></tr>
<tr><td colspan="3" rowspan="3">Participants</td><td colspan="3">Kyriakos Spanoudes</td></tr>
<tr><td colspan="3">Theocharis Drakos</td></tr>
<tr><td colspan="3">Nikolas Evripidou</td></tr>
<tr><td colspan="3">Principal Investigator</td><td colspan="3">Christakis Damianou</td></tr>
<tr><td colspan="6"></td></tr>
<tr><td colspan="3">Time</td><td colspan="3">Anesthesia</td></tr>
<tr><td colspan="3"></td><td colspan="3"></td></tr>
<tr><td colspan="3">Euthanasia</td><td colspan="3"></td></tr>
<tr><td colspan="6"></td></tr>
<tr><td>Time</td><td>Heart Rate</td><td>Resp. Rate</td><td>Urination
(0 to 3)</td><td>Absence of Movement/Pedal Reflex</td><td>Temp. (°C)</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td></tr>
</table>

During the experiments, proper care of the experimental animals was taken. All experimental animals were treated in scientifically and humanely appropriate ways, according to the guidelines provided by the Animal Welfare Committee.

Evaluation of the robotic system (version 1) in rabbits

Materials and Methods

The 4 degrees of freedom (DOF) robotic device (version 1) was used for the experiments conducted on the first 20 rabbits. The ultrasonic and motion parameters were controlled through the developed MRI-guided focused ultrasound (MRgFUS) software (Deliverable 5.1). The software was connected to the electronic driving system (Deliverable 3.3) that actuates motion in the axes. This version of the robotic device includes small piezoelectric motors (USR30-S3N, Shinsei Corporation, Kasuya Setagaya-ku, Tokyo, Japan), therefore the corresponding electronic driving system that hosts the drivers for small motors was used. The robotic device guides a 1.1 MHz transducer (MEDSONIC LTD, Limassol, Cyprus) with 50 mm diameter, 70 mm radius of curvature, and an efficiency of 31 %. The piezoceramic element was purchased from the Piezo Hannas Tech Co, LTD (Wuhan City, HuBei Province, China). An amplifier (AG1016, T & C Power Conversion, Inc., Rochester, USA) was used to apply high power to the focused transducer. A schematic diagram of the experimental set-up is shown in Figure 1 while a photo of the experimental set-up using the first version of the robotic device is shown in Figure 2.

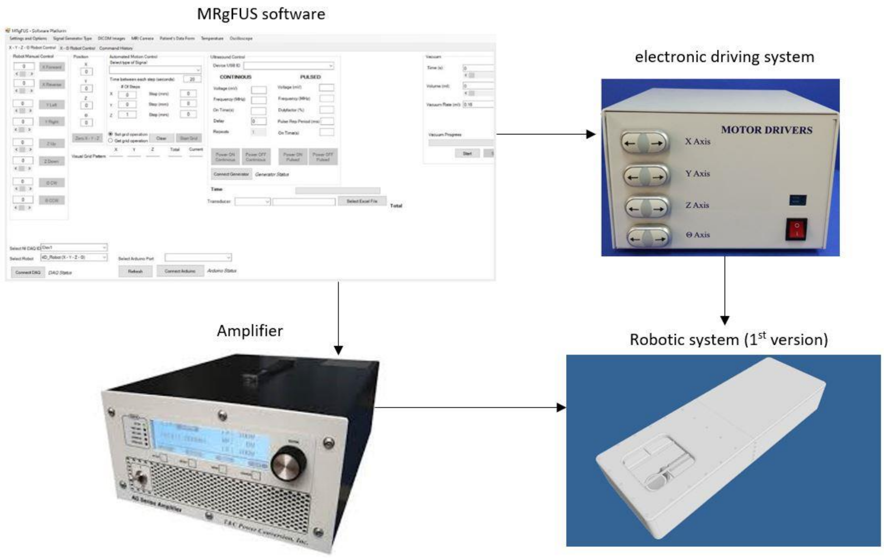

Figure 1: Schematic diagram of the experimental set-up using the 1st version of the 4-DOF robotic device.

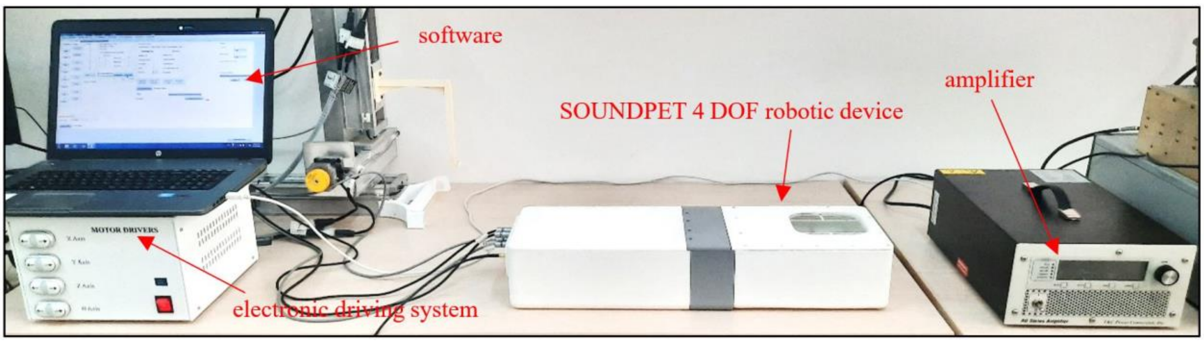

Figure 2: Experimental set-up using the 1st version of the 4-DOF robotic device.

Locally bred rabbits purchased from an accredited vendor (S. Ioannou) were transferred to the premises of the therapeutic ultrasound laboratory (Cyprus University of Technology, Limassol, Cyprus), examined and weighed. All rabbits underwent injectable anesthesia with a combination of medetomidine (1-1.5 ml) and ketamine (0.3-0.5 ml). The initial dose was given according to the weight of each rabbit. This drug combination offers 40-60 minutes of surgical anesthesia, which can be prolonged with additional smaller doses (up to half of the initial dose).

Before the experiments, the thighs of the rabbit were depilated (VEET, Reckitt Benkiser, Slough, UK). Then, the rabbit was placed on the acoustic window of the robotic device with its thigh immersed in the degassed water, above the transducer. High Intensity Focused Ultrasound (HIFU) sonications were performed on the outer side of the thighs (left and right) of each rabbit. A water-filled plastic bag was placed between each rabbit's thighs with extra ultrasound gel added between the plastic bag/thigh interface to eliminate any reflections arising from possible tissue/air interface. X-rays were taken prior to HIFU sonications utilising an X-ray system (IMS001, Shenzhen Browiner Tech Co., Ltd, Shenzhen, China). The purpose of the X-ray was to calculate from the image the muscle area available for ablation, thus avoiding sonicating the bone. The animal was placed on left recumbency above a CR (Computed Radiography) cassette. After X-ray exposure, a CR reader (Vita Flex, Carestream Health, Inc., 150 Verona Street, Rochester, NY, USA) was used to convert the data into a digital image. Following HIFU sonications, ultrasound examination of the thigh was performed using a portable ultrasound device (DP-50, Mindray, Shenzhen, China) with a linear probe (frequency range 5-10 MHz). Upon completion of the experimental procedure, the animals were humanely euthanized with intracardial injection of an agent containing embutramide, mebezonium, and tetracaine (T61, MSD Animal Health, New Jersey, USA). Carcasses were temporarily stored in a -20 °C freezer and transferred to the premises of SIGAN management LTD (7735 Kofinou, Larnaca, Cyprus) for appropriate disposal. Figure 3 demonstrates the stages of animal preparation and configuration for HIFU sonications.

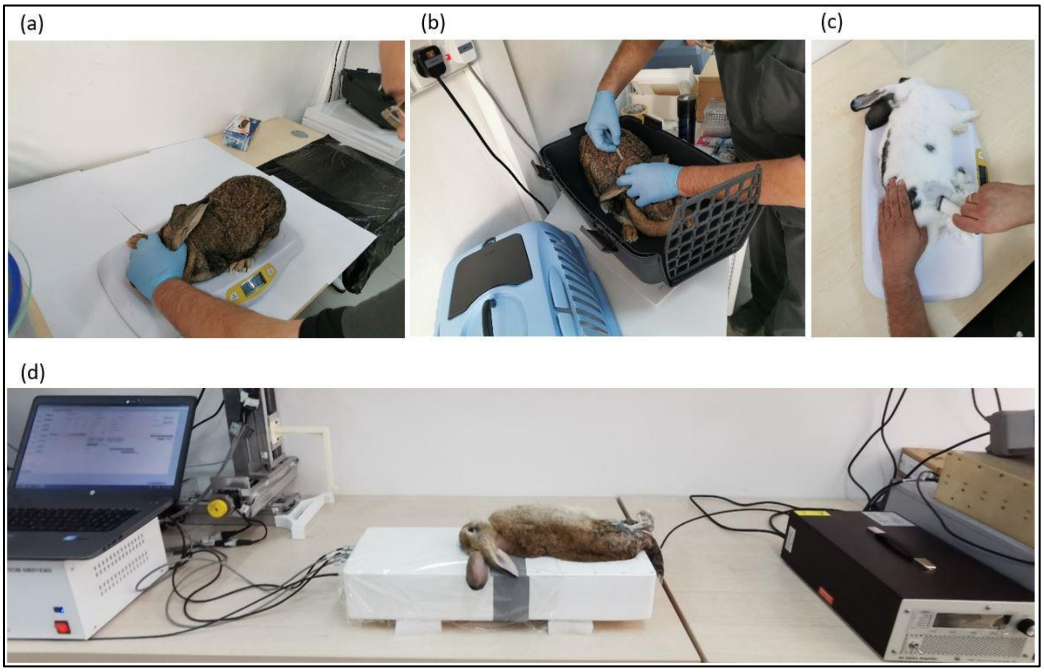

Figure 3: Animal preparation for ablation of the thighs, (a) weighing, (b) injection of the anesthesia, (c) thighs shaving, and (d) placement of the anesthetized rabbit on the robotic device.

HIFU sonications were executed with varied ultrasonic parameters to observe the effect of the sonication parameters on the dimensions (length and diameter) of the formed discrete lesions or the size of the ablated area created by overlapping lesions. For each experiment executed on each rabbit thigh, a table with the main ultrasonic parameters used was completed. The template of the table is shown in Table 2.

Table 2: Sample of the ultrasonic settings table that was completed for each rabbit thigh and experiment.

Parameter	Value
Electric power (W)	
Acoustic power (W)	
Sonication time (s)	
Energy (J)	
Cooling time (s)	
Number of sonications	
Step between sonications (mm)	
Distance transducer/rabbit skin (cm)	

The evaluation of version 1 of the robotic device was performed following distinctive motion grid patterns as shown in Figure 4. In each experiment, motion of the robotic device followed one of the selected grid patterns with different step movements (spatial step) to achieve either discrete or overlapping lesions. A cooling period was allowed between successive sonications for eliminating near-field heating.

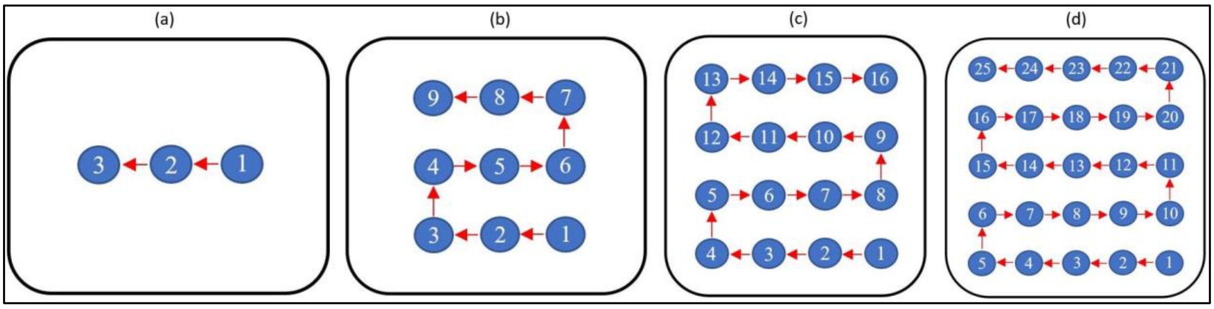

Figure 4: Grid patterns followed with the 1st version of the robotic device, a) 1 × 3, b) 3 × 3, c) 4 × 4, and d) 5 × 5. The spatial step varied depending on the location of the multiple sonications to create discrete or overlapping lesions.

The concept of the rabbit experiment is shown in Figure 5. An acoustic window on the top cover of the system allows transmission of ultrasound from the transducer to the target through the degassed water which is used as a coupling agent. The rabbit was positioned on the acoustic window in a way that allowed exposure of the thigh to the ultrasonic beam. Robotic motion was performed in such a way that the transducer remained in the available sonication window thus ensuring that all sonications were focused on the rabbit thigh. In this regard, the maximum grid pattern that could be followed was 5 × 5 which could be used for ablating a large muscle area.

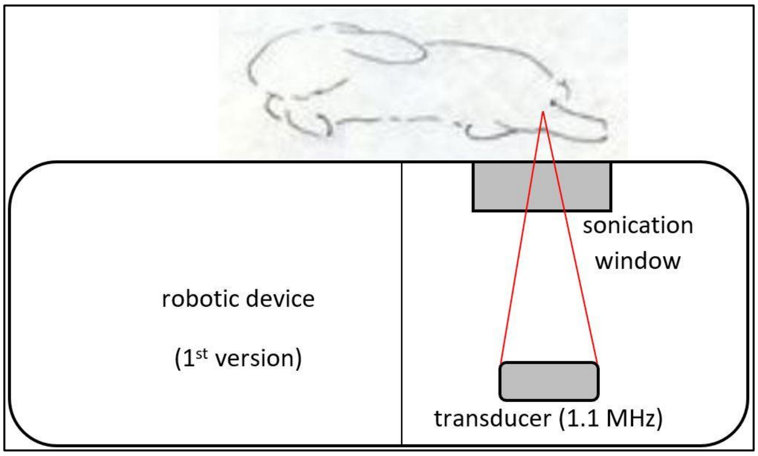

Figure 5: Abstract representation of the experimental setting.

Results

Prior to treatment, the purpose of X-ray was to calculate the muscle area available for ablation, thus avoiding sonications of the bone. A sample of an X-ray image of a rabbit thigh is demonstrated in Figure 6. Acquisition of this image allowed demarcation of the muscle area available for ablation and decision-making regarding the number of sonications that would be followed (i.e. grid pattern size).

Figure 6: X-ray image taken from a rabbit to allow calculation of thigh muscle area for appropriate selection of grid pattern and treatment plan. The muscle area for this example was 24.02 cm^2.

Prior to and following HIFU sonications, ultrasound examination of the rabbit thigh was performed using a portable ultrasound device (DP-50, Mindray) with a linear probe. Figure 7 and Figure 8 show the ultrasound image of the rabbit thigh muscle taken prior to and following sonications, respectively.

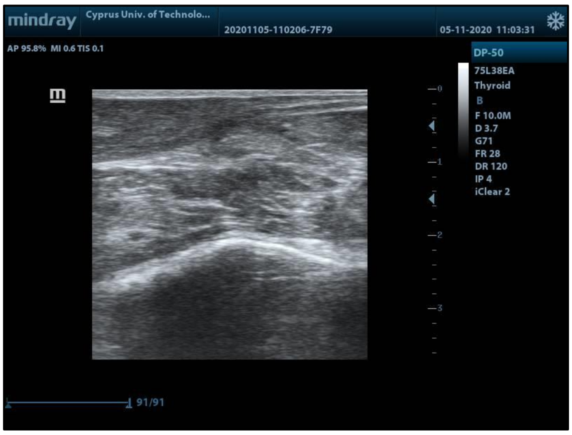

Figure 7: Ultrasound image of the rabbit thigh muscle acquired prior to sonications.

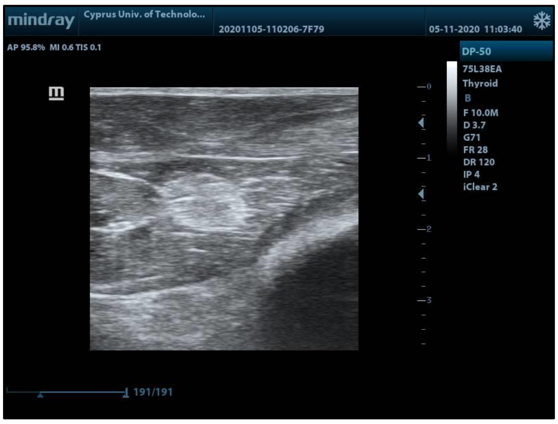

Figure 8: Ultrasound image of the rabbit thigh muscle acquired following sonications.

For each rabbit thigh, following sonications, photos were taken on the inner and outer sides of the thigh above and below the skin as well as upon exposure of the ablated muscle. Photos obtained upon exposure of the ablated muscle showed the formed discrete lesions or the area created by overlapping lesions on a plane perpendicular to the ultrasound beam. Additionally, photos were obtained after cross-section thus showing the lesions or ablated area on a plane parallel to the ultrasound beam. Figure 9 demonstrates discrete lesions formed with the SOUNDPET robotic system version 1, using varied sonication parameters and following varied grid patterns. These lesions are on a plane perpendicular to the ultrasound beam.

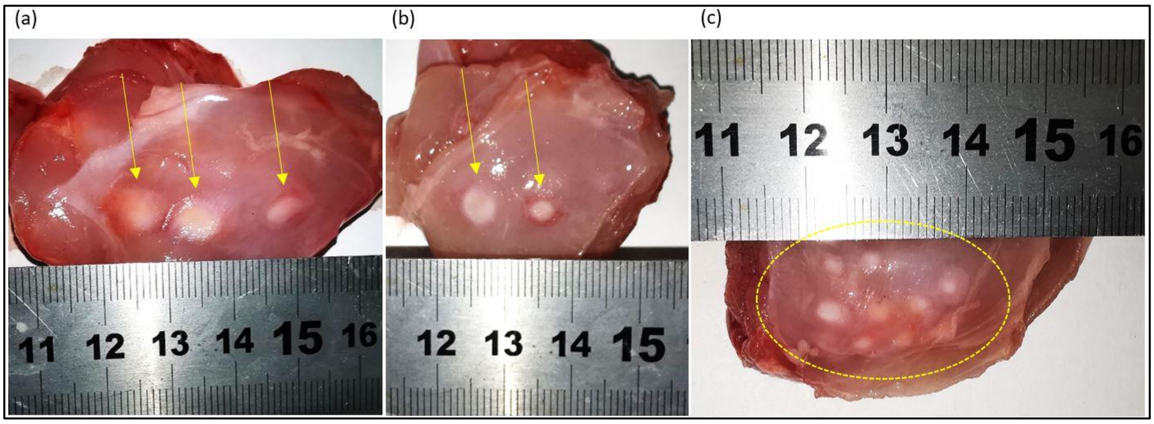

Figure 9: Sample images of discrete lesions inflicted by ablation with the SOUNDPET system (version 1) on a plane perpendicular to the ultrasound beam (f = 1.1 MHz, D = 50 mm, R = 70 mm) for a grid pattern of (a, b) 1 × 3, and (c) 4 × 4. The yellow arrows and circles indicate the discrete lesions.

Figure 10 demonstrates the corresponding discrete lesions, after cross-section, on a plane parallel to the ultrasound beam.

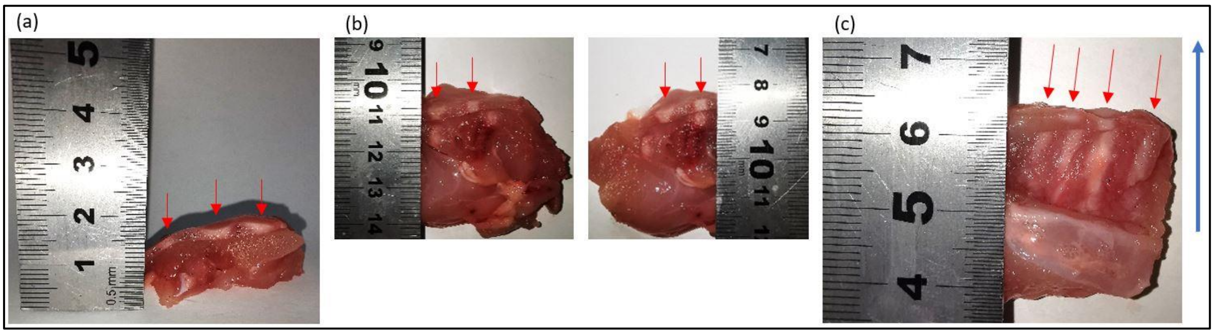

Figure 10: Sample images after cross-section of discrete lesions inflicted by ablation with the SOUNDPET system (version 1) on a plane parallel to the ultrasound beam (f = 1.1 MHz, D = 50 mm, R = 70 mm) for a grid pattern of (a, b) 1 × 3, and (c) 4 × 4. The red arrows indicate the discrete lesions and the blue arrow indicates the ultrasonic beam direction.

Overlapping lesions were formed by using a small step movement between each sonication. A higher number of sonications (larger motion grid pattern) was performed for ablating a large muscle area. Figure 11 demonstrates overlapping lesions on a plane perpendicular to the ultrasound beam inflicted after ablation performed with the robotic system by employing a range of sonication parameters and grid patterns.

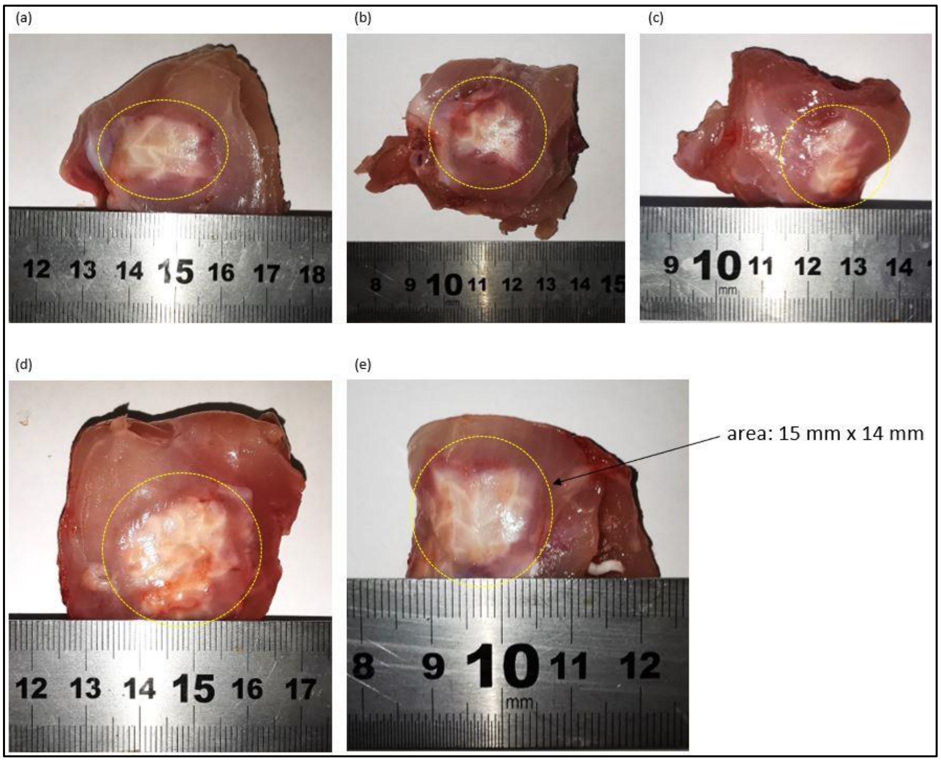

Figure 11: Sample images of overlapping lesions inflicted by ablation with the SOUNDPET system (version 1) on a plane perpendicular to the ultrasound beam (f = 1.1 MHz, D = 50 mm, R = 70 mm) for a grid pattern of (a, b, c) 3 × 3, (d) 4 × 4, and (e) 5 × 5. The yellow circles indicate the ablated area.

Figure 12 demonstrates overlapping lesions, after cross-section, inflicted by ablation with the robotic system (version 1) on a plane parallel to the ultrasound beam.

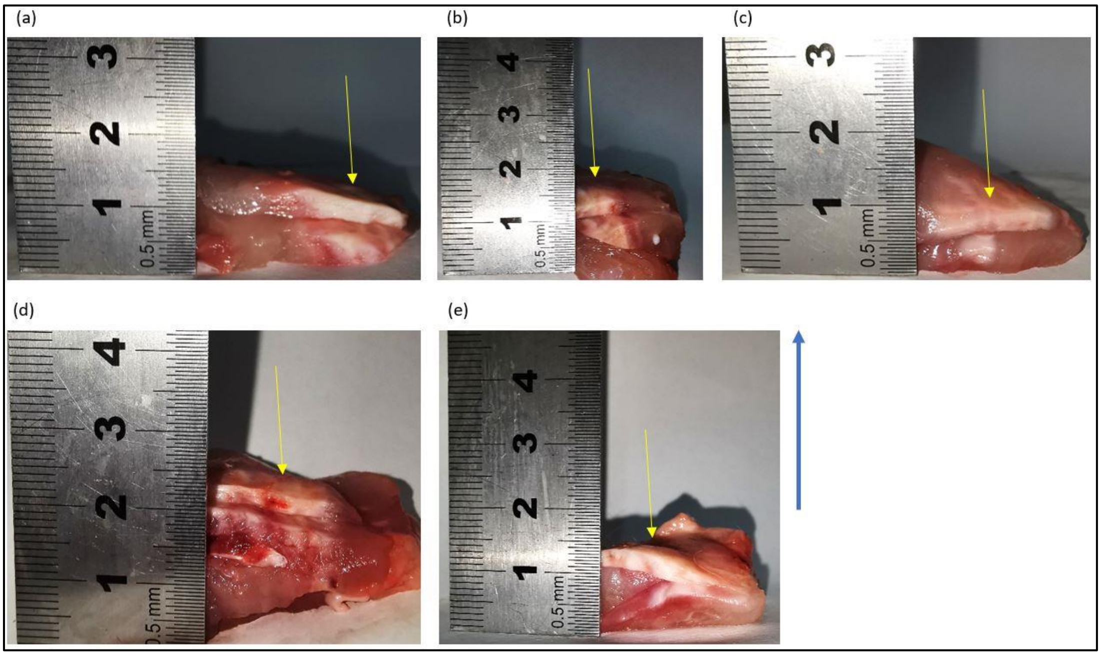

Figure 12: Sample images after cross-section of overlapping lesions inflicted by ablation with the SOUNDPET system (version 1) on a plane parallel to the ultrasound beam (f = 1.1 MHz, D = 50 mm, R = 70 mm) for a grid pattern of (a, b, c) 3 × 3, (d) 4 × 4, and (e) 5 × 5. The yellow arrows indicate the ablated area and the blue arrow indicates the ultrasonic beam direction.

Table 3 summarizes the range of the main experimental parameters used in the *in vivo* experiments including the sonication time, acoustic power, step movement and grid pattern as well as the range of dimensions of the formed discrete and overlapping lesions. Table 4 lists the detailed ultrasonic and robotic motion settings for each experiment per rabbit thigh and the outcome of the ablation.

Table 3: List of parameters used in the *in vivo* rabbit experiments performed with the robotic system (version 1).

Parameters	Values range	
Sonication time (s)	3 - 20	
Acoustic power (W)	62 - 108.5	
Energy (J)	279 - 1240	
Step movement (mm)	4 - 10	
Grid pattern	1 × 3 - 5 × 5	
Discrete lesions	**Diameter (mm)**	**Length (mm)**
	3 - 10	1 - 17
Overlapping lesions	**Area (mm^2)**	**Depth (mm)**
	19 × 9 - 30 × 26	2 – 17

Table 4: Detailed list of the ultrasonic and robotic motion settings for each ablation per rabbit thigh using the SOUNDPET robotic system (version 1) and the potential of the transducer (f = 1.1 MHz, D = 50 mm, R = 70 mm) to produce lesions with the corresponding settings.

Rabbit	Thigh (L/R)	Step movement (mm)	Ultrasonic parameters				Grid	Number of formed lesions	Lesion diameter (mm)	Lesion length (mm)
			Distance transducer/ rabbit (cm)	Acoustic power (W)	Sonication time (s)	Energy (J)				
1	L	10	6	62	20	1240	1 × 3	Overlapping area	25 × 5	5
	R	10	6	62	20	1240	1 × 3	2	8, 6	5, 5
2	L	10	6	62	10	620	1 × 3	2	5, 7	4, 3
	R	10	7	62	10	620	1 × 3	3	5, 8, 5	-, 6, 4
3	L	10	7	62	5	310	1 × 3	3	6, 5, 5	8, 4, -
	R	10	7	62	10	620	1 × 3	2	4, 3	17, 4
4	L	10	7	62	10	620	1 × 3	2	7, 5	10, 6
	R	10	7	77.5	5	387.5	1 × 3	3	6, 6, 5	3, 1, 2
5	L	10	7	62	5	310	1 × 3	-	-	-
	R	10	7	62	10	620	1 × 3	3	8, 8, 7	3, 2, 2
6	L	10	7	77.5	5	387.5	1 × 3	3	5, 5, 5	6, 2, -
	R	10	7	77.5	10	775	1 × 3	2	5, 4	11, 8
7	L	5	7	62	10	620	3 × 3	Overlapping area	19 × 15	10
	R	5	7	62	5	310	3 × 3	4	5, 4, 4, 3.5	5, 6, 3, 1
8	L	5	7	77.5	10	775	3 × 3	Overlapping area	16 × 14	8
	R	5	7	77.5	5	387.5	3 × 3	4 (faint)	-	-
9	L	5	7	93	10	930	3 × 3	Overlapping area	15 × 15	7
	R	5	7	93	5	465	3 × 3	Overlapping area	19 × 9	2
10	L	5	7	93	10	930	3 × 3	Overlapping area	15 × 17	14
	R	5	7	77.5	10	775	3 × 3	Overlapping area	13 × 12	8
11	L	5	7	93	5	465	4 × 4	Overlapping area	19 × 15	8

	R	5	7	93	3	279	4 × 4	Overlapping area (some lesions discrete)	22 × 19	11
12	L	4	7	93	5	465	4 × 4	Overlapping area	25 × 21	13
	R	4	7	93	3	279	4 × 4	Overlapping area	21 × 22	7
13	L	4	8	93	5	465	4 × 4	Overlapping area	26 × 11	11
	R	4	8	93	3	279	4 × 4	10	3, 2.5, 2.5, 4, 5, 4, 2, 4, 3, 2.5	4, 10, 12, 8, 11.5, 9, 8, 10, 7, 6
14	L	5	7	93	5	465	5 × 5	Overlapping area	15 × 14	7
	R	5	7	93	5	465	5 × 5	Overlapping area	24 × 25	10
15	L	10	7	93	5	465	1 × 3	3	7, 8, 9	8, 4, 8
	R	10	7	93	10	930	1 × 3	3	8, 4, 4.5	8, 4, 2
16	L	10	7	93	10	930	1 × 3	1	4	-
	R	10	7	93	5	465	1 × 3	3	6, 4, 3	2, 5, 3
17	L	10	7	108.5	5	542.5	1 × 3	3	10, 6, 3	3, 3, -
	R	10	7	108.5	3	325.5	1 × 3	2	4, 4	10, -
18	L	5	7	108.5	5	542.5	4 × 4	Overlapping area	25 × 13	13
	R	5	7	108.5	3	325.5	4 × 4	Overlapping area	18 × 18	11
19	L	5	7	93	5	465	5 × 5	Overlapping area	30 × 26	10
	R	4	7	93	3	279	5 × 5	Overlapping area	20 × 20	4
20	L	5	7	108.5	5	542.5	5 × 5	Overlapping area	23 × 15	17
	R	4	7	108.5	3	325.5	5 × 5	Overlapping area	17 × 17	5

Discussion

The 1st version of the SOUNDPET robotic system has shown capable of creating tissue necrosis in a controllable and reproducible manner as it has been proven through its *in vivo* evaluation process in healthy small animals (rabbits). In all 20 rabbits, ablation remained in the targeted zone (muscle thigh area) without damaging the surrounding tissue neither injuring tissue in areas outside of the original sonication planning, thus suggesting the ability of creating necrosis in a controllable manner without any unwanted side-effects. Handling of the experimental animals following the guidelines provided by the Animal Welfare Committee ensured that there was no indication of animal suffering since all animals remained in deep general anesthesia until euthanasia.

The low-frequency (1.1 MHz) transducer has the ability to ablate tissue situated deep in the body without affecting healthy intermediate tissue. In the majority of the rabbit thighs, no lesions were observed on the outer side of the thigh (side of ultrasonic beam propagation) since the formed lesions (discrete or overlapping ablated area) were only visible on the inner thigh. This finding is well in accordance with the focal length (70 mm) of the transducer and indicates that coupling (with a water-filled plastic bag, placed between the thighs of the rabbit) facilitated efficient ablation.

The length of the discrete lesions varied from superficial to 17 mm, while their diameter as measured from the exposed muscles varied from 3 to 10 mm. Thermal discrete lesions were formed during the experiments, indicating the ability of the transducer to create lesions in a safe and reproducible manner without the occurrence of any unwanted effects. Compared to the *in vitro* experiments (Deliverable 6.3), the occurrence of cavitation was greatly reduced since there are no bubbles in the living rabbit tissue. Large grid patterns were performed with small step movements and necrosis in the form of overlapping lesions was created at the target muscle area as expected. Depending on the applied ultrasonic energy and the size of the grid pattern, overlapping lesions resulted in the creation of ablated regions whose area ranged from 19 × 9 mm to 30 × 26 mm while their length varied from superficial (1 mm) to 17 mm.

A minimum ultrasonic energy of 279 J was sufficient for creation of discrete or overlapping lesions. The ideal scenario for future clinical practices of the proposed system is setting the sonication time below 5 s for creating thermal lesions thus minimizing the time required for the entire procedure and HIFU treatment. Minimization of the sonication time with simultaneous application of high ultrasonic energy (in the form of high acoustic power) provides some other advantages including lower doses of patient anesthesia and less time of transducer activation that leads to less transducer stress and therefore longer operating life. The transducer with application of appropriate acoustic power has the advantage to create lesions with a sonication time of 3 s.

As observed from the 20 experiments, a total treatment time of 26 minutes and 15 seconds was required to ablate an area of 17×17 mm^2 by performing movement in a 5 × 5 grid pattern. It was also concluded that the 60 s interval allowed between successive sonications is sufficient to allow cooling of the transducer and eliminate pre-heating for adjacent sonications.

No bugs were detected in the software upon connection and throughout operation with the robotic system, thereby allowing accurate movement in all axes and precise transducer control.

A main shortcoming of the small ultrasonic motors utilised in version 1 of the robotic system is that in case they remain inactive for a long period they block and manual restoration is needed. In this regard, a 2nd version robotic device was designed and developed to address this issue. The 2nd version uses the large piezoelectric motors of the same company (Shinsei Corporation) which are more reliable.

Evaluation of the robotic system (version 2) in rabbits

Materials and Methods

The 4-DOF robotic device (version 2) (Deliverable 3.1) was used for the experiments performed on the second set of 20 rabbits. The ultrasonic and robotic motion parameters were controlled through the developed MRgFUS software (Deliverable 5.1). Similarly with the *in vivo* experiments performed with version 1 of the robotic system, the software was connected to the electronic driving system for initiating and controlling robotic motion. However, since motion of version 2 of the robotic device is carried out through large piezoelectric motors (USR60-S3N, Shinsei Corporation), the electronic driving system for this robotic device differs in the drivers. The robotic device guides a 2.6 MHz transducer (MEDSONIC LTD) with 38 mm diameter, 61 mm radius of curvature, and an efficiency of 23 %. The piezoelectric element was purchased from the Piezo Hannas Tech Co, LTD. The amplifier (AG1016, T & C Power Conversion, Inc.) was used to apply high power to the focused transducer. A schematic diagram of the experimental set-up is shown in Figure 13, while the experimental set-up with the second version of the robotic device is shown in Figure 14.

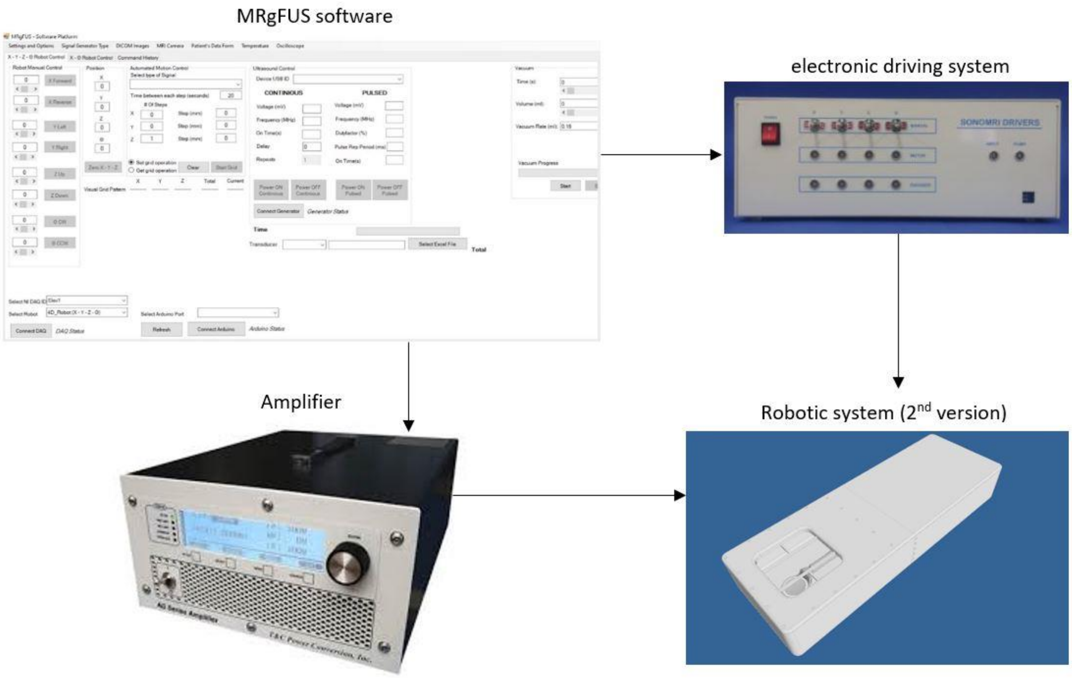

Figure 133: Schematic diagram of the experimental set-up using the 4-DOF robotic device (2nd version).

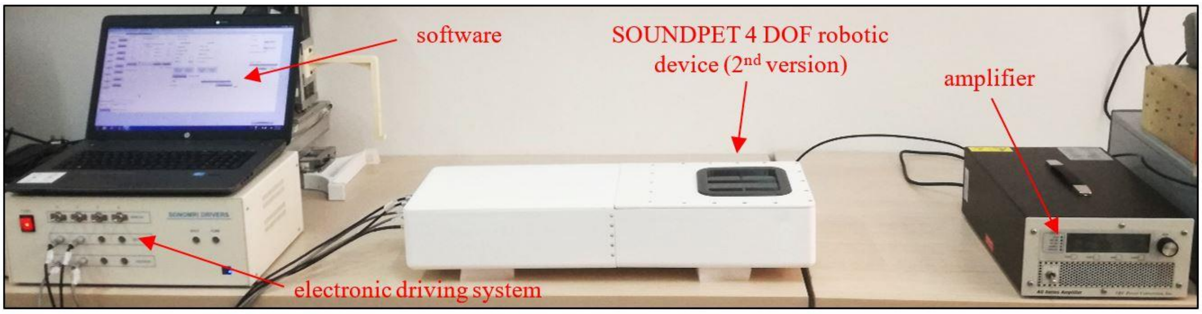

Figure 14: Experimental set-up using the 4-DOF robotic device (2nd version).

Locally bred rabbits were purchased from an accredited vendor (S. Ioannou) and transferred to the premises of the therapeutic ultrasound laboratory (Cyprus University of Technology, Limassol, Cyprus), examined and weighed. All rabbits underwent injectable anesthesia with a combination of medetomidine (1-1.5 ml) and ketamine (0.3-0.5 ml) which offers 40-60 minutes of surgical anesthesia, which can be prolonged with smaller doses (up to half of the initial dose). A water-filled plastic bag was placed between the thighs of each rabbit with additional ultrasound gel on the plastic bag/thigh interface to eliminate any reflections returning from possible tissue/air interface. For the last eight rabbits, only the ultrasound gel was added between the rabbit thighs without placement of the water-filled plastic bag to investigate the possibility of the ultrasound beam propagating through the ultrasound gel and ablating the other non-sonicated thigh. The thighs of the first rabbit (21) were used for optimization of the transducer settings and the motion of the robotic device.

After optimization of the transducer and motion settings, sonications on all other rabbits were performed using varied ultrasonic parameters for observing their influence on the dimensions of the formed discrete lesions or the area of the ablated region created by overlapping lesions. Following the same procedure undertaken in the first set of 20 rabbits, a table (Table 5) with the main ultrasonic parameters was completed for each experiment conducted on each rabbit thigh.

Table 5: Sample of the ultrasonic settings table that was completed for each rabbit thigh and experiment.

Parameter	Value
Electric power (W)	
Acoustic power (W)	
Sonication time (s)	
Energy (J)	
Cooling time (s)	
Number of sonications	
Step between sonications (mm)	
Distance transducer/rabbit skin (cm)	

For the second set of 20 rabbit experiments executed for evaluation of version 2 of the robotic device, the following grid patterns (Figure 15) were performed. In each experiment, robotic motion followed one of the selected grid patterns with varied step movements so that sonications would result in discrete or overlapping lesions. A cooling period was allowed between movements for eliminating pre-heating from adjacent sonications.

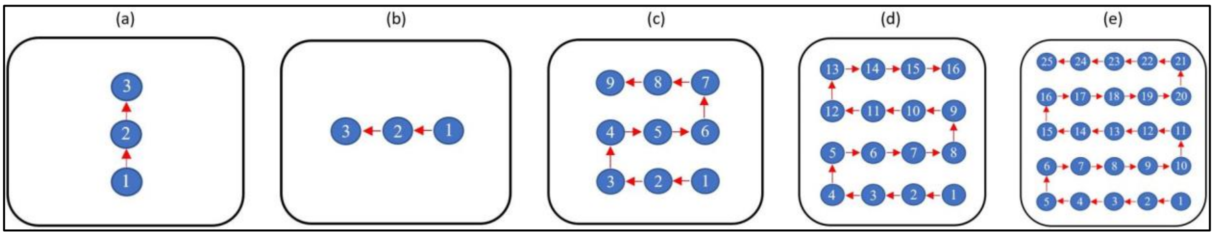

Figure 15: Grid patterns followed for the second set of 20 rabbit experiments performed with the 2nd version of the robotic device, a) 1 × 3 (motion in the Y axis), b) 1 × 3 (motion in the X axis), c) 3 × 3, d) 4 × 4, and e) 5 × 5. The spatial step varied depending on the location of the multiple sonications to create discrete or overlapping lesions.

The concept of the rabbit experiment is shown in Figure 16. The experiments followed an identical concept with the setting used in experiments performed with the 1st version of the robotic device. The rabbit was placed in a way that allowed thigh exposure to the ultrasonic beam. In this regard, in the 2nd version of the robotic device, a sonication window existed on the cover of the system to allow propagation of the ultrasound beam from the transducer to the rabbit thigh through the degassed water (coupling agent). The position of the transducer was set to remain in the available sonication window throughout the experimental procedure, therefore robotic motion could be performed in a maximum grid pattern of 5 × 5 which was used for ablations of large areas.

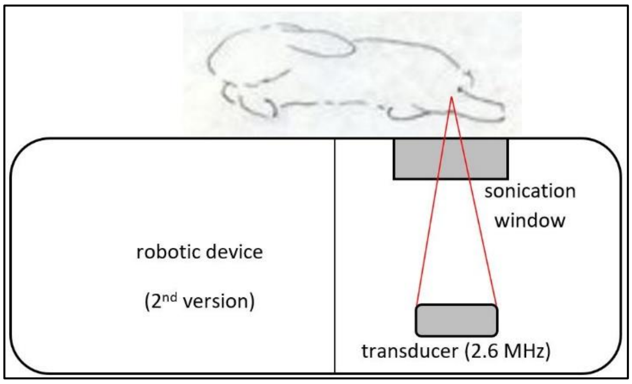

Figure 16: Abstract representation of the experimental setting.

Results

Subsequent to sonications, for each rabbit thigh, a photo was taken on the inner and outer sides of the thigh above and below the skin as well as when the ablated muscle was exposed. Photos of the exposed ablated muscle showed the formed discrete lesions or the ablated region created by overlapping lesions on a plane perpendicular to the ultrasound beam. Photos obtained upon cross-section of the lesions or ablated area indicated necrosis formed on a plane parallel to the ultrasound beam. Figure 17 demonstrates discrete lesions formed with the SOUNDPET robotic system version 2 using different sonication and motion parameters. These lesions are on a plane perpendicular to the ultrasound beam.

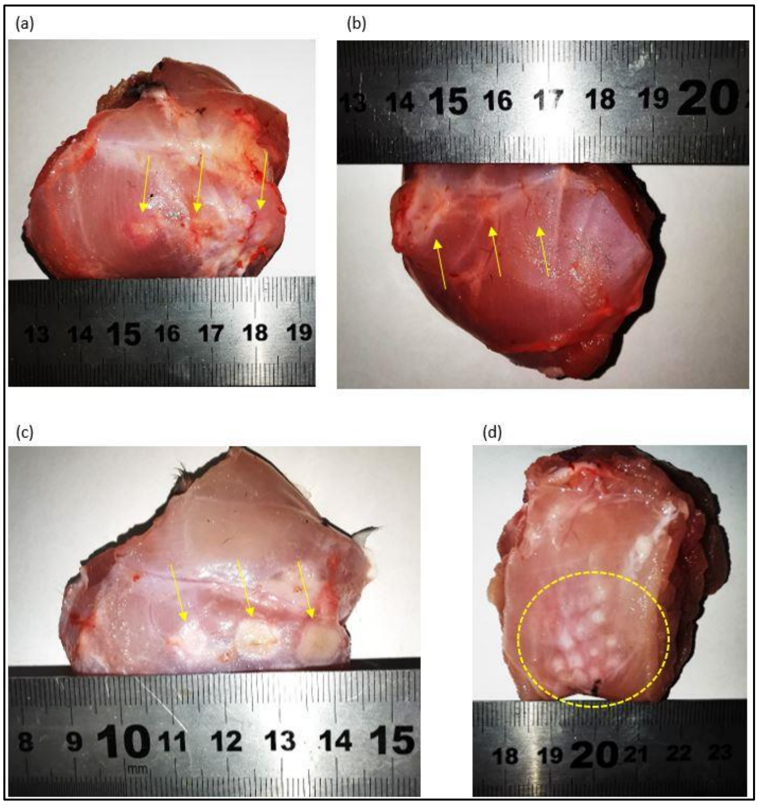

Figure 17: Photos of discrete lesions inflicted by ablation with the SOUNDPET system (version 2) on a plane perpendicular to the ultrasound beam (f = 2.6 MHz, D = 38 mm, R = 61 mm) for a grid pattern of (a, b, c) 1 × 3, and (d) 4 × 4. The yellow arrows and circles indicate the discrete lesions.

Figure 18 demonstrates the corresponding discrete lesions, after cross-section, on a plane parallel to the ultrasound beam.

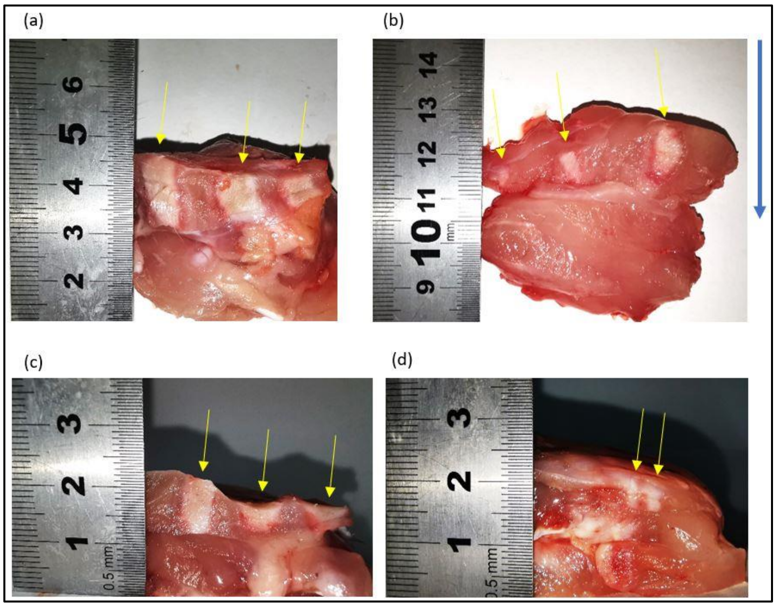

Figure 18: Photos after cross-section of discrete lesions inflicted by ablation with the SOUNDPET system (version 2) on a plane parallel to the ultrasound beam (f = 2.6 MHz, D = 38 mm, R = 61 mm) for a grid pattern of (a, b, c) 1 × 3, and (d) 4 × 4. The yellow arrows indicate the discrete lesions and the blue arrow indicates the direction of the ultrasonic beam.

Overlapping lesions were additionally formed by minimizing the spatial step of robotic motion performed between each sonication. A higher number of sonications (larger grid patterns) was performed for ablating larger muscle areas. Figure 19 demonstrates overlapping lesions on a plane perpendicular to the ultrasound beam inflicted by ablation with the robotic system version 2 using varied sonication and motion settings.

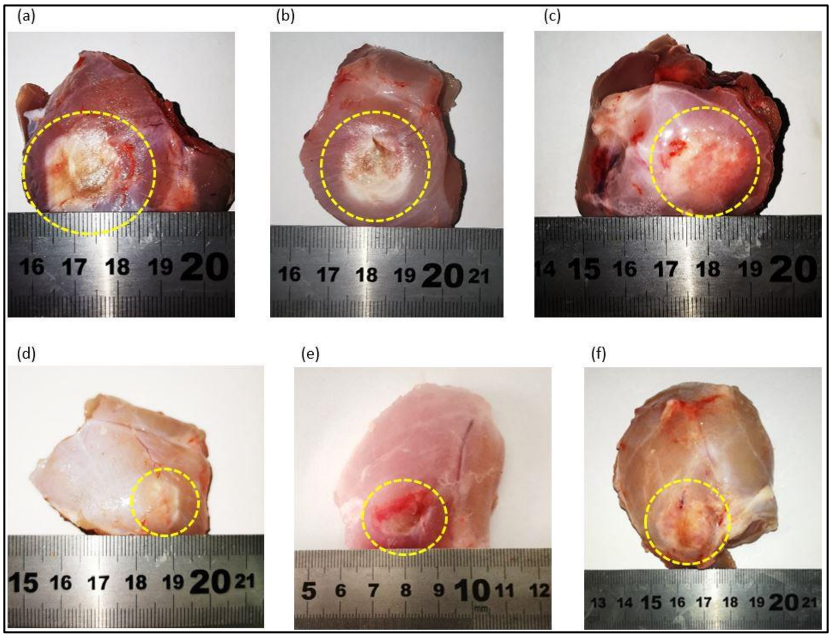

Figure 19: Photos of overlapping lesions inflicted by ablation with the SOUNDPET system (version 2) on a plane perpendicular to the ultrasound beam (f = 2.6 MHz, D = 38 mm, R = 61 mm) for a grid pattern of (a, b, d) 3 × 3, (c, e) 4 × 4, and (f) 5 × 5. The yellow circles indicate the ablated area.

Figure 20 demonstrates the overlapping lesions, after cross-section, on a plane parallel to the ultrasound beam inflicted by ablation with the robotic system (version 2).

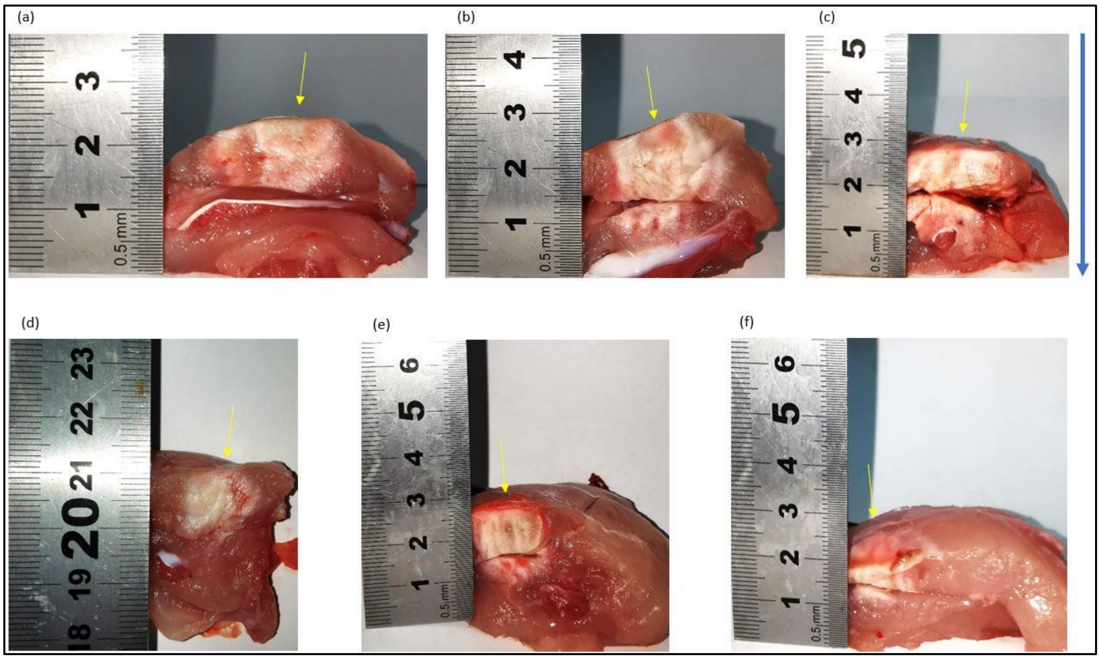

Figure 20: Photos, after cross-section of overlapping lesions inflicted by ablation with the SOUNDPET system (version 2) on a plane parallel to the ultrasound beam (f = 2.6 MHz, D = 38 mm, R = 61 mm)

for a grid pattern of (a, b, d) 3 × 3, (c, e) 4 × 4, and (f) 5 × 5. The yellow arrows indicate the length of the ablated area and the blue arrow indicates the ultrasonic beam direction.

Table 6 summarizes the range of the main experimental parameters used in the second set of 20 rabbits employed for the *in vivo* evaluation the robotic system (version 2), including the sonication time, acoustic power, step movement, and grid pattern. The range of dimensions of the formed discrete and overlapping lesions is also provided.

Table 6: List of parameters used in the *in vivo* rabbit experiments performed with the robotic system (version 2).

Parameters	Values range	
Sonication time (s)	3 - 30	
Acoustic power (W)	23 - 69	
Energy (J)	172.5 - 1380	
Step movement (mm)	3 - 15	
Grid pattern	1 × 1 - 5 × 5	
Discrete lesions	**Diameter (mm)**	**Length (mm)**
	2 - 11	2 - 16
Overlapping lesions	**Area (mm^2)**	**Depth (mm)**
	7 × 11 - 24 × 20	4 - 19

Table 7 lists the ultrasonic settings and robotic motion parameters used in each *in vivo* experiment per rabbit thigh and the outcome of the ablation.

Table 7: Detailed list of the ultrasonic and robotic motion settings for each ablation per rabbit thigh using the SOUNDPET robotic system (version 2) and the potential of the transducer (f = 2.6 MHz, D = 38 mm, R = 61 mm) to produce lesions with the corresponding settings.

Rabbit	Thigh (L/R)	Step movement (mm)	Ultrasonic parameters				Grid	Number of created lesions	Lesion diameter (mm)	Lesion length (mm)
			Distance transducer/rabbit (cm)	Acoustic power (W)	Sonication time (s)	Energy (J)				
21	L	-	5	-	-	-	-	-	-	-
	R	-	5	46	30	1380	1 × 1	1	5	7
22	L	15	5	23	30, 20, 10	690, 460, 230	1 × 3	2	6, 4	11, 9
	R	15	5	23	30, 20, 10	690, 460, 230	1 × 3	2	9, 7	16, 14
23	L	15	5	34.5	30, 20, 10	1035, 690, 345	1 × 3	1	6	9
	R	15	5	34.5	30, 20, 10	1035, 690, 345	1 × 3	3	6, 5, 8	5, 7, 8
24	L	15	5	34.5	20, 10, 5	690, 345, 172.5	1 × 3	1	4	2
	R	15	5	34.5	20, 10, 5	690, 345, 172.5	1 × 3	2	4, 3	8, 4
25	L	15	5	34.5	30, 20, 10	1035, 690, 345	1 × 3	1	6	2
	R	15	5	34.5	30, 20, 10	1035, 690, 345	1 × 3	2	6, 9	9, 5
26	L	15	5	46	20, 10, 5	920, 460, 230	1 × 3	3	8, 7, 3	8, 4, -
	R	15	5	46	20, 10, 5	920, 460, 230	1 × 3	2	8, 7	9, 8
27	L	15	5	46	10	460	1 × 3	3	6, 6, 4	14, 8, 6
	R	15	5	46	5	230	1 × 3	3	4, 3, 2	13, 8, 5
28	L	15	5	57.5	20, 10, 5	1150, 575, 287.5	1 × 3	3	9, 8, 7	3, 4, 10
	R	15	5	57.5	20, 10, 5	1150, 575, 287.5	1 × 3	3	11, 7, 2	8, 7, 2
29	L	4	5	46	20	920	3 × 3	Overlapping area	19 × 19	12
	R	4	5	46	10	460	3 × 3	Overlapping area	19 × 20	19
30	L	4	5	46	5	230	3 × 3	Overlapping area	14 × 10	15

	R	4	5	46	10	460	3 × 3	Overlapping area	15 × 15	11
31	L	4	5	57.5	10	575	4 × 4	2 overlapping areas	20 × 14, 14 × 11	17
	R	4	5	57.5	5	287.5	4 × 4	Overlapping area	16 × 6	4
32	L	4	5	57.5	5	287.5	4 × 4	Overlapping area	19 × 15	17
	R		5	57.5	3	172.5	4 × 4	14	3 (average)	4 (average)
33	L	4	5	57.5	5	287.5	3 × 3	Overlapping area	9 × 12	6
34	L	3	5	57.5	3	172.5	3 × 3	Overlapping area	7 × 14	14
35	L	4	5	57.5	5	287.5	3 × 3	Overlapping area	7 × 11	16
36	L	3	5	57.5	3	172.5	3 × 3	Overlapping area	12 × 12	5
37	L	4	5	69	5	345	3 × 3	Overlapping area	20 × 18	12
38	L	3	5	69	3	207	4 × 4	Overlapping area	16 × 14	18
39	L	3	5	69	3	207	5 × 5	Overlapping area	20 × 20	8
40	L	3	5	69	5	345	5 × 5	Overlapping area	24 × 20	7

Discussion

The 2nd version of the SOUNDPET robotic system has shown capable of creating necrosis in a controllable and reproducible manner as it has been proven through its evaluation process in healthy small animals (rabbits). In all 20 rabbits, ablation of tissue remained in the targeted zone (muscle thigh area), without damaging the surrounding tissue, other intervening areas or regions outside of the treatment planning, thereby suggesting no unwanted side-effects. There was no indication of animal suffering and all animals remained in deep general anesthesia until euthanasia.The 2.6 MHz frequency transducer that was incorporated in the 2nd version of the robotic system has the ability to ablate tissue shallower than the transducer (1.1 MHz) of the previous version. In the majority of the thighs, lesions (discrete or overlapping ablated region) were visible only on the outer thigh (side of sonication) with no lesions formed on the inner side of the thigh. This is in accordance with the focal length of the transducer and suggests that coupling in the form of a water-filled plastic bag placed between the thighs facilitated efficient ablation. In the last eight rabbits where only ultrasound gel was added between the thighs without placement of the water-filled bag, no lesions appeared on the non-sonicated thigh indicating controllability of the ablations since these did not induce damage in other areas.

Higher grid patterns were performed using the software and necrosis at the target muscle area was created as expected.

The length of the discrete lesions varied from 2 to 16 mm, while their diameter as measured from the exposed muscles varied from 2 to 11 mm. Equivalent to lesions created with the transducer in the 1st version of the robotic system, experiments with the higher frequency transducer resulted in the formation of thermal discrete lesions. Ablated regions created by overlapping lesions were formed with areas that ranged from 7 × 11 mm to 24 × 20 mm while their length varied from 4 mm to 19 mm, depending on the applied ultrasonic energy. These findings suggest that the robotic system can safely ablate surface tissues and tumors with a maximum depth of 20 mm. The ability of the system to ablate tumors in companion animals (dogs and cats) is presented in Deliverable 6.6.

A minimum ultrasonic energy of 172.5 J created discrete and overlapping lesions. Compared to the focused transducer used in the previous version, this transducer creates lesions with less ultrasonic energy due to its smaller focal beam (higher ultrasound intensity produced). The transducer with the appropriate application of acoustic power has the advantage to create lesions with a sonication time of 3 s.

Compared to experiments performed with the transducer in the previous version, a total treatment time of 26 minutes and 15 seconds resulted in ablation of a larger area of 20 × 20 mm^2 when performing movement in a 5 × 5 grid pattern. Despite the higher frequency of this transducer, the 60 s interval allowed between sonications was again sufficient to allow cooling of the transducer and eliminate pre-heating of adjacent sonications.

In *in vivo* experiments performed with the 2nd version of the robotic system, no bugs were detected in the software upon its connection and operation with the system thus allowing accurate control of the robotic motion and ultrasonic parameters. The use of large motors in this version of the robotic system overcame the issue of motors block after a lengthy period of inactivation observed in the 1st version of the system.

Appendix 1 – SOUNDPET robotic system 1st version – Rabbit *in vivo* experiments (1-20)

1. Purpose

To assess the ability of the SOUNDPET robotic device to ablate the thigh of a living rabbit, find the minimum ultrasonic energy that is needed to create thermal lesions in rabbit tissue and test the movement accuracy of the robotic device.

2. Experimental materials and procedure

2.1 Experimental materials

The following experimental materials were used:

- Amplifier: AG1016 (AG Series Amplifier, T & C Power Conversion, Inc., Rochester, US)
- Tissue: Thigh (Live Rabbit)
- Transducer: ID 34 (frequency: 1.1 MHz, diameter: 50 mm, radius of curvature: 70 mm, efficiency: 31 %)
- SOUNDPET 4 DOF robotic system
- Electronic driving system
- MRgFUS software.

2.2 *In vivo* experiment

All experiments were approved by the authorities of the Veterinary Services, Ministry of Agriculture (CY/EXP/PR.L01/2020). Locally bred rabbits (purchased from an accredited farm; S. Ioannou-CYCU5.05) were examined, weighed, and anesthetized (see anesthesia record in each individual report). The thighs of each rabbit were depilated (VEET, Reckitt Benkiser, UK) shaved and each time the rabbit was placed on the robotic device with its thigh immersed in the degassed water above the transducer. The sonications were performed on the outer side of the thighs (left and right) of each rabbit.

3. Individual reports

Rabbit 1

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	200
Acoustic power (W)	62
Sonication time (s)	20
Energy (J)	1240
Cooling time (s)	60
Number of sonications	3
Step between sonications (mm)	10
Distance transducer/rabbit skin (cm)	6

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved reverse from point 1 to 3 at step movement of 10 mm and the distance between the transducer and the opening sonicated window of the robotic device was 6 cm.

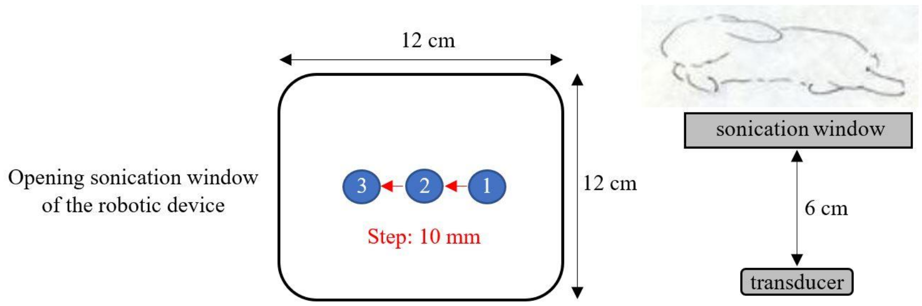

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	200
Acoustic power (W)	62
Sonication time (s)	20
Energy (J)	1240
Cooling time (s)	60
Number of sonications	3
Step between sonications (mm)	10
Distance transducer/rabbit skin (cm)	6

A linear grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved reverse from point 1 to 3 at step movement of 10 mm and the distance between the transducer and the opening sonicated window of the robotic device was 6 cm.

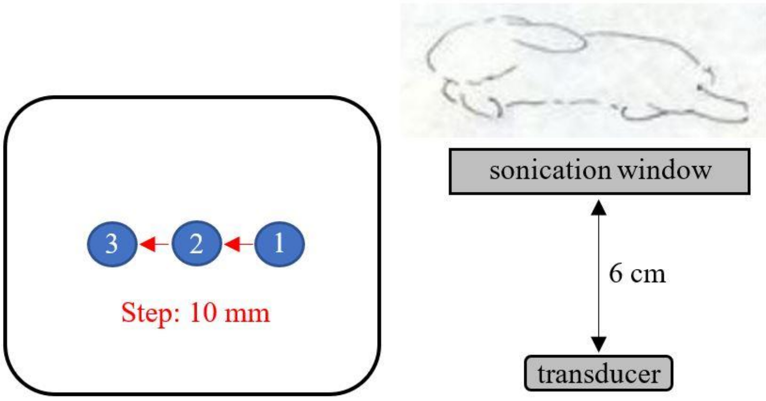

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory that was used for the rabbit thigh ablations.

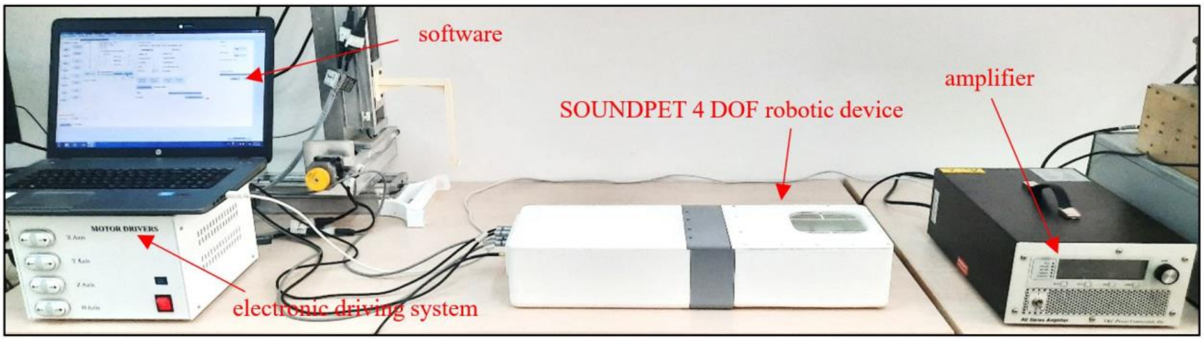

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

Figure 4: Animal positioning for the experiment.

Results

As shown in Figure 5 and Figure 6, no lesions were appeared from the side of the sonication (outer side) on the left thigh above and below the skin, respectively.

Figure 5: The outer side of the left thigh above the skin after the treatment.

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side).

Necrotic area was observed on the inner side of the left thigh both above and below the skin as shown in Figure 7 and Figure 8, respectively.

Figure 7: Macroscopic appearance of the lesions from the inner side of the left thigh and above the skin. The red arrows indicate the formed lesions on the skin.

The formed lesions were appeared merely discrete above the skin while below the skin they were overlapped by creating a necrotic region of around 25 mm.

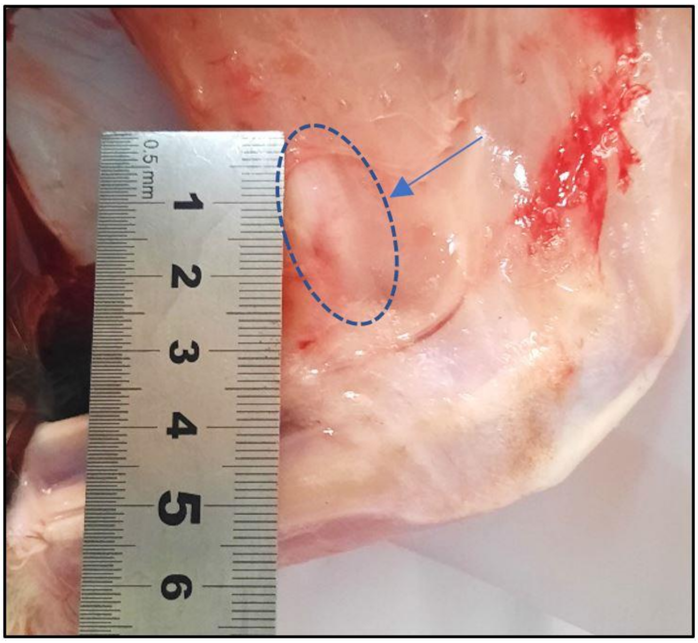

Figure 8: Macroscopic appearance of the overlapping lesions on the exposed muscles from the inner side of the left thigh. The blue arrow indicates the created necrotic region after performing a linear grid of 3 sonications at step movement of 10 mm.

The depth of the necrotic area was calculated to be 5 mm while 5 mm of the muscle remained unaffected (from the side of the sonications). Figure 9 demonstrates the dissected tissue of the rabbit muscle of the left thigh.

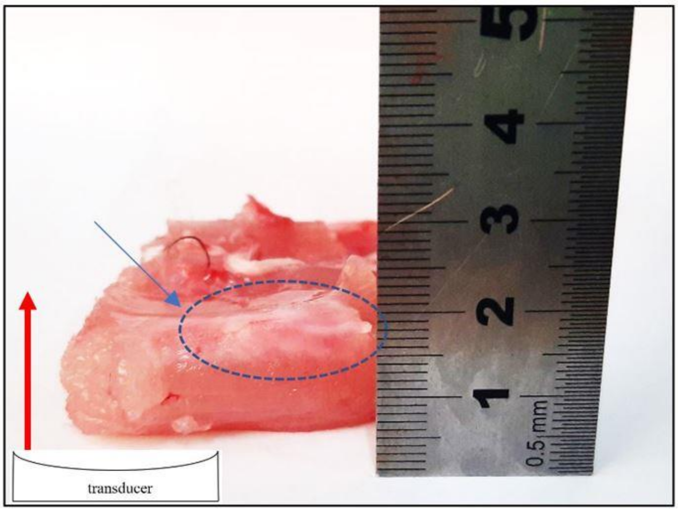

Figure 9: In situ dissection of the necrotic area of the left thigh. The blue arrow indicates the formed necrotic area and the red arrow indicates the beam direction.

As shown in Figure 10, no lesions were appeared on the right thigh above and below the skin, respectively. However, the lesions were palpable on the skin.

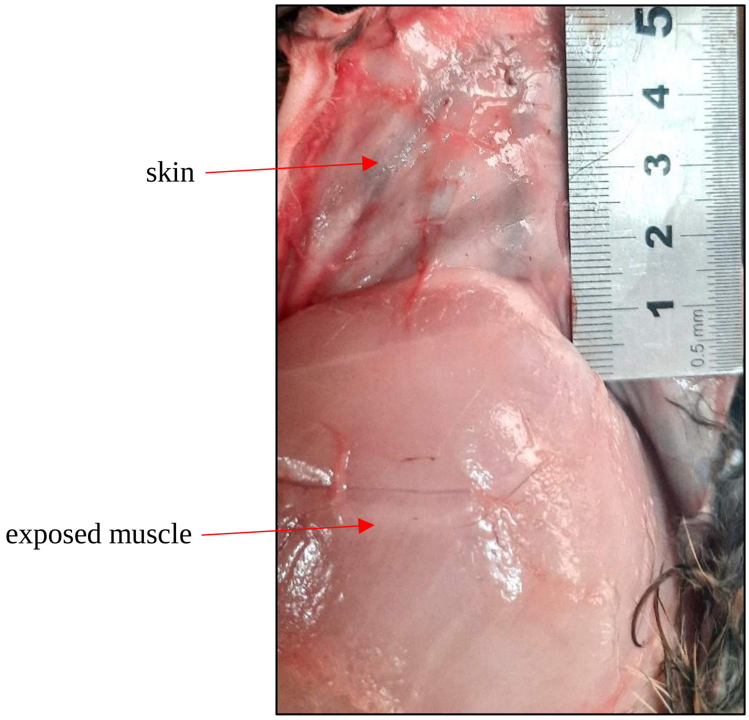

Figure 10: The outer side of the right thigh following treatment and when the skin was detached from the muscle.

The muscle of the right thigh was dissected and overlapping lesions were appeared as shown in Figure 11. The depth of the ablation was calculated to be at 1 cm from the skin surface.

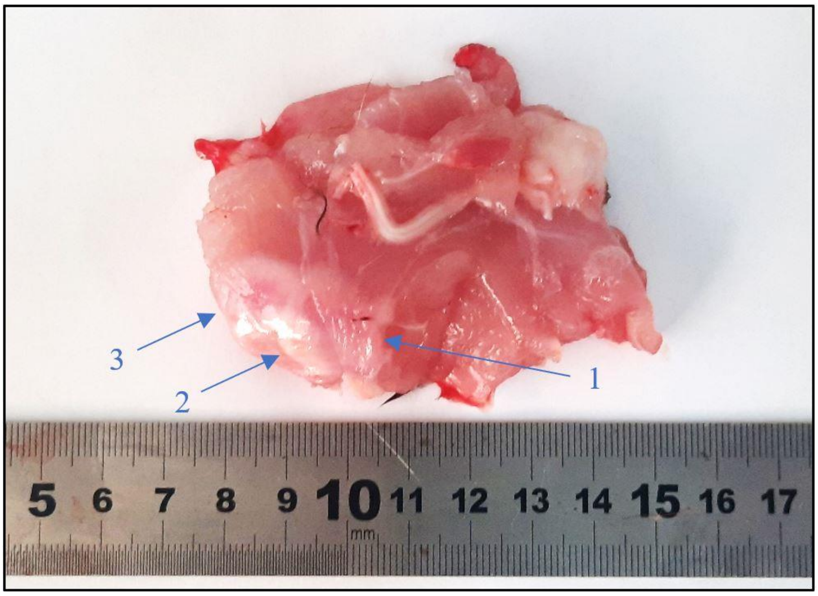

Figure 11: Macroscopic appearance of the formed lesions (indicated with blue arrows) on the dissected muscle of the right thigh (plane perpendicular to the beam).

The depth of the necrotic area was calculated to be 5 mm and it was formed at the surface of the muscle. Figure 12 demonstrates the dissected tissue of the rabbit muscle of the right thigh.

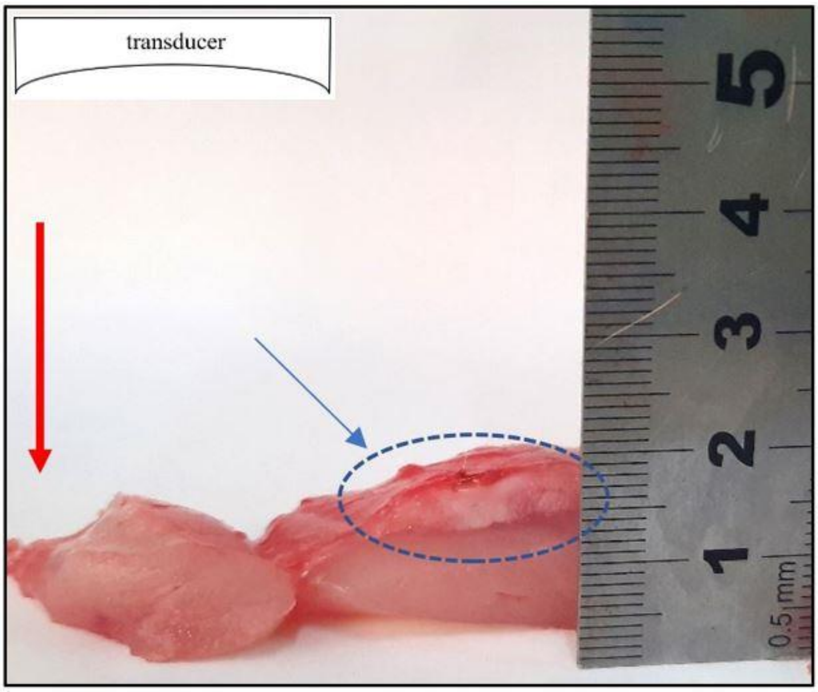

Figure 12: In situ dissection of the necrotic area of the right thigh. The blue arrow indicates the formed necrotic area and the red arrow indicates the beam direction.

Three (3) lesions were observed on the right thigh after the treatment (Table 3). The diameter and length of the two lesions was measurable.

Table 3: Summary of results for the right thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	8	5
2	6	5
3	-	-

Table 4 lists the anesthesia record for the rabbit experiment.

Table 4: Anesthesia record for rabbit 1.

Animal ID	SOUNDPET_2020_1
Species	Rabbit
Sex	M
Age	Adult
Weight (Kg)	2.85
Supplier	S. Ioannou Farm (CYCU5.05)
Grant	SOUNDPET (INTEGRATED/0918/0008)
Study	CY/EXP/PR.L01/2020
Participants	Kyriakos Spanoudes

	Theocharis Drakos
	Nikolas Evripidou
Principal Investigator	Christakis Damianou
Time	**Anesthesia**
11:00	Medetomidine 1.5 ml + Ketamine 0.45 ml
11:30	Medetomidine 1 ml + Ketamine 0.2 ml
Euthanasia	11:45 1 ml T-61

Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
11:00	110	45	-	Absent	n/a
11:15	120	50	-	Absent	n/a
11:30	120	45	-	Absent	n/a
11:45	110	45	-	Absent	n/a

Rabbit 2

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	200
Acoustic power (W)	62
Sonication time (s)	10
Energy (J)	620
Cooling time (s)	60
Number of sonications	3
Step between sonications (mm)	10
Distance transducer/rabbit skin (cm)	6

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved reverse from point 1 to 3 at step movement of 10 mm and the distance between the transducer and the opening sonicated window of the robotic device was 6 cm.

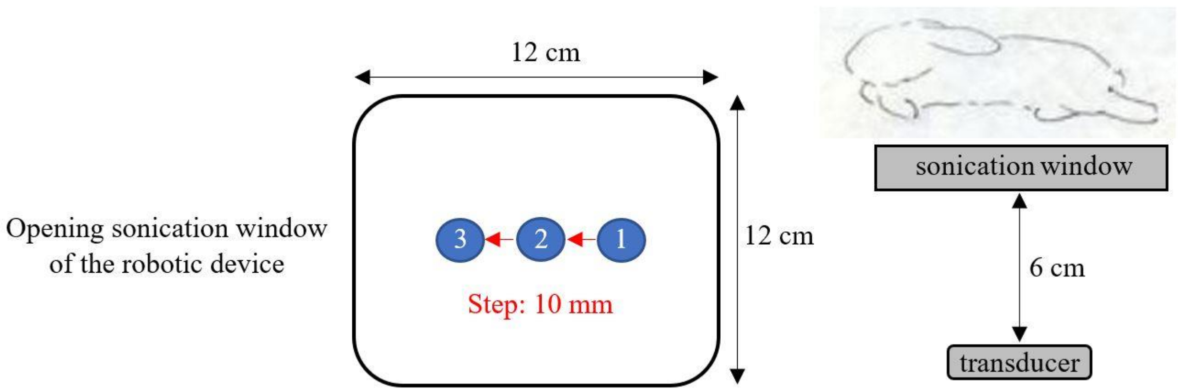

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	200
Acoustic power (W)	62
Sonication time (s)	10
Energy (J)	620
Cooling time (s)	60
Number of sonications	3
Step between sonications (mm)	10
Distance transducer/rabbit skin (cm)	7

A linear grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved reverse from point 1 to 3 at step movement of 10 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

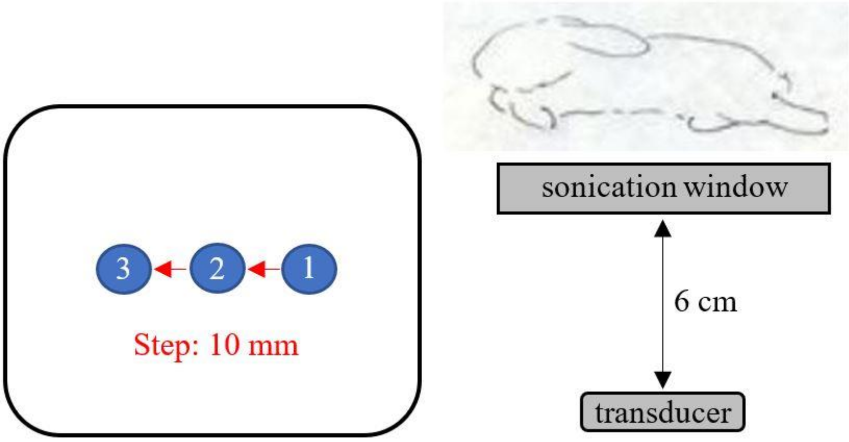

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory, that was used for the rabbit thigh ablations.

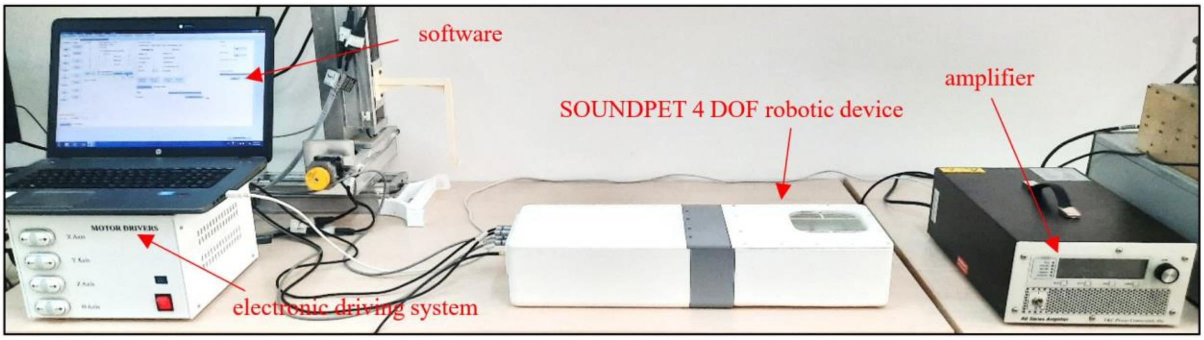

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

Figure 4: Animal positioning for the experiment.

Results

As shown in Figure 5 and Figure 6, no lesions were appeared on the outer side (sonication side) of the left thigh above and below the skin, respectively.

Figure 5: The outer side of the left thigh above the skin after the treatment.

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side).

Necrotic area was observed on the inner side of the left thigh both above and below the skin as shown in Figure 7 and Figure 8, respectively. The formed lesions were appeared discrete below the skin.

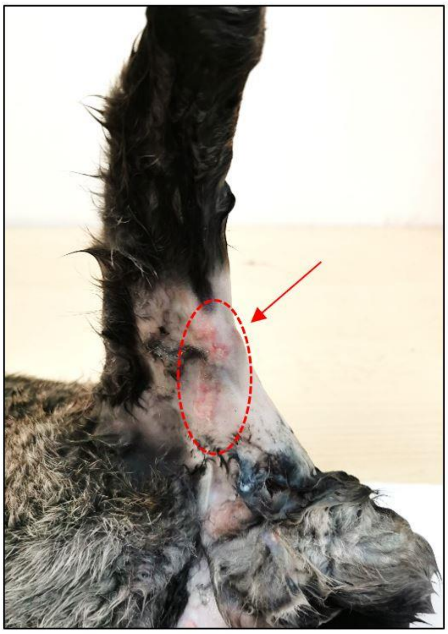

Figure 7: Macroscopic appearance of the lesions from the inner side of the left thigh and above the skin. The red arrow indicates the necrotic area on the skin.

Figure 8: Macroscopic appearance of the discrete lesions on the exposed muscles from the inner side of the left thigh. The red arrows indicate the formed lesions after performing a linear grid of 3 sonications at step movement of 10 mm.

The muscle of the left thigh was dissected and discrete lesions were appeared as shown in Figure 9.

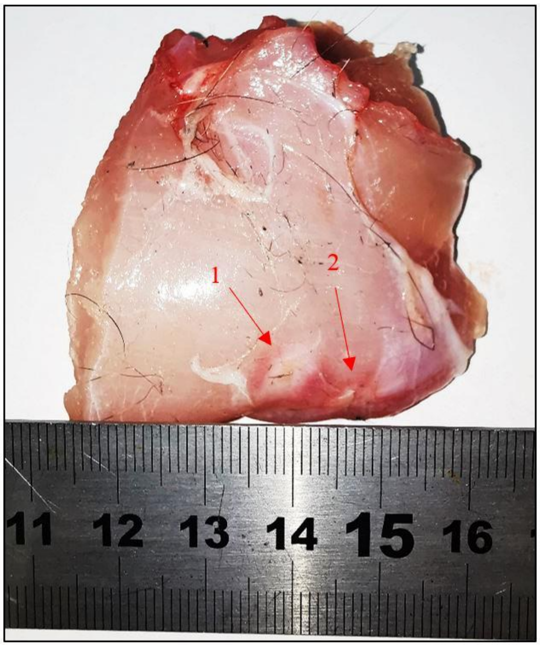

Figure 9: Macroscopic appearance of the formed lesions (indicated with red arrows) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The depth of the lesion 1 and 2 was calculated to be 5 mm and 3 mm, respectively while 5 mm of the muscle remained unaffected (from the sonication side). Figure 10 and Figure 11 demonstrates findings after dissection of lesion 1 and lesion 2 formed at the left thigh, respectively.

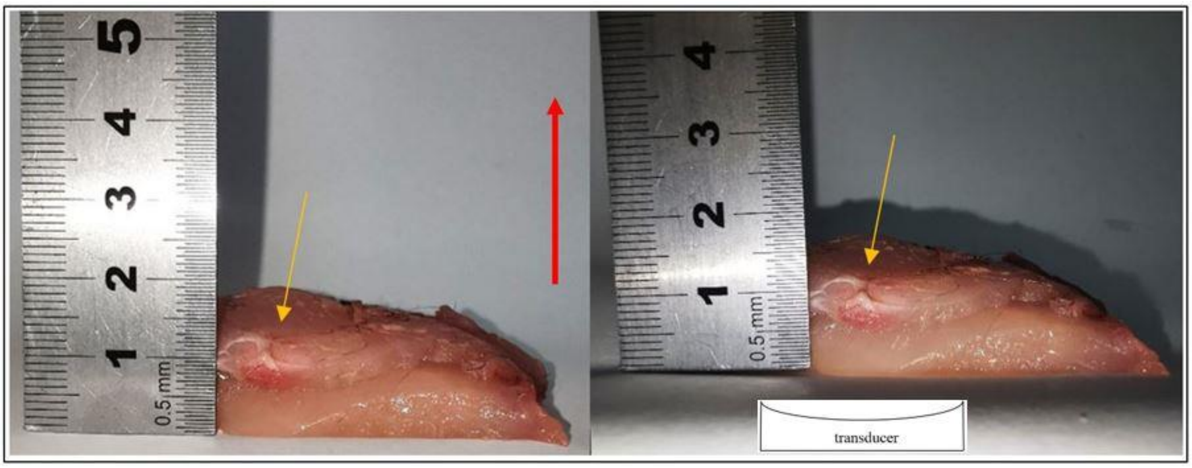

Figure 10: In situ dissection of the lesion 1 of the left thigh. The yellow arrow indicates the formed lesion and the red arrow indicates the beam direction.

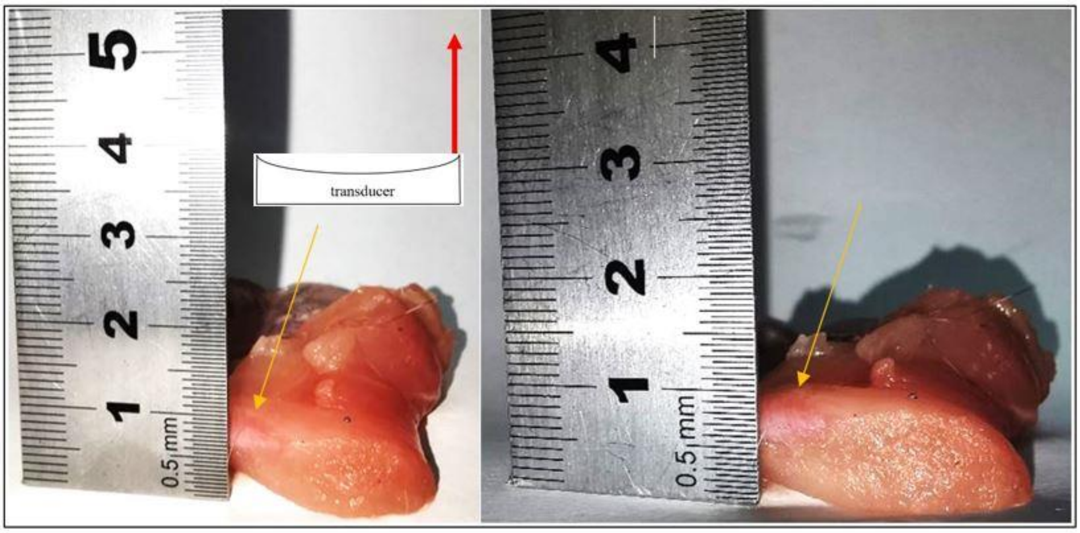

Figure 11: In situ dissection of the lesion 2 of the left thigh. The yellow arrow indicates the formed lesion and the red arrow indicates the beam direction.

Two (2) lesions were observed on the left thigh after the treatment (Table 3). The diameter and length of the two lesions was measurable.

Table 3: Summary of results for the left thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	5	4
2	7	3
3	-	-

As shown in Figure 12 and Figure 13, no lesions were appeared on the right thigh above and below the skin from the side of sonication, respectively.

Figure 12: The right thigh following treatment above the skin from the side of the sonication.

Figure 13: The right thigh following treatment after the skin was removed and the muscle was exposed from the side of the sonication.

Discrete lesions were formed on the inner side of the right thigh both above and below the skin as shown in Figure 14 and Figure 15, respectively.

Figure 14: Macroscopic appearance of the lesions from the inner side of the right thigh and above the skin. The red arrows indicate the discrete lesions that were appeared on the skin.

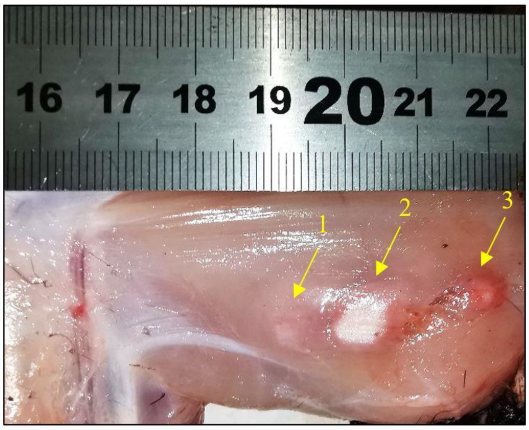

Figure 15: Macroscopic appearance of the discrete lesions on the exposed muscles from the inner side of the right thigh. The yellow arrows indicate the formed lesions after performing a linear grid of 3 sonications at step movement of 10 mm.

The muscle of the inner side of the right thigh was dissected and three (3) discrete lesions were appeared as shown in Figure 16 and one lesion was appeared on the outer side of the right thigh as shown in Figure 17.

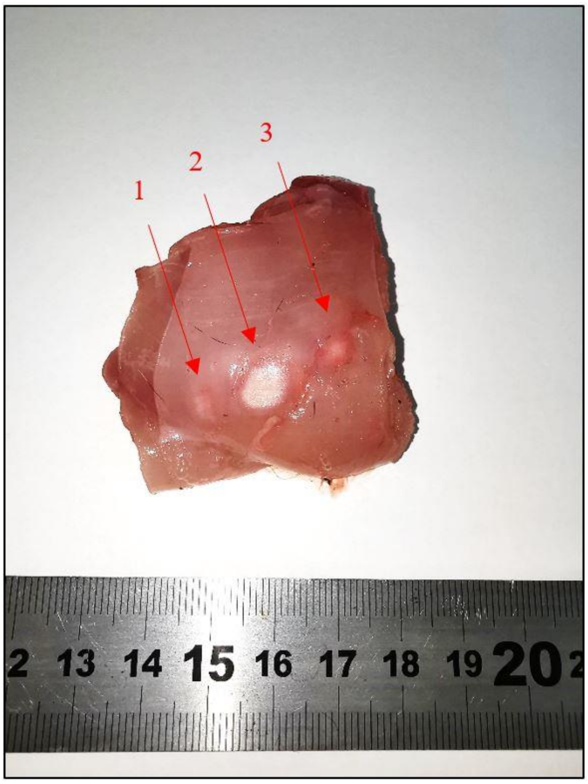

Figure 16: Macroscopic appearance of the formed lesions (indicated with red arrows) on the dissected muscle of the inner side of the right thigh (plane perpendicular to the beam).

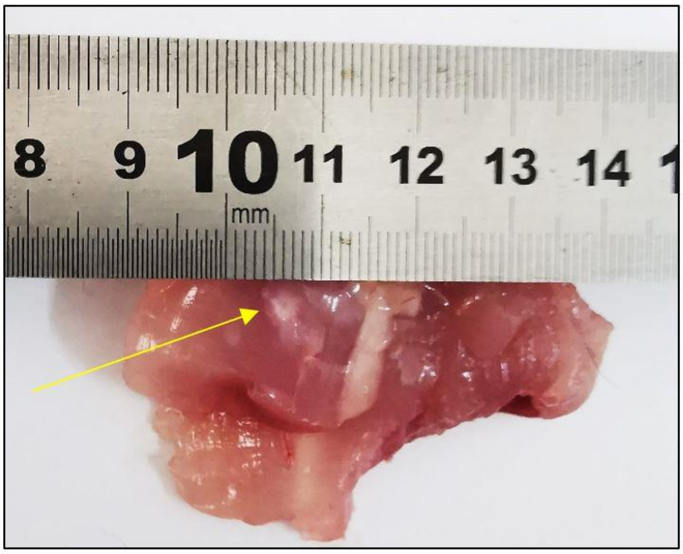

Figure 17: Macroscopic appearance of the formed lesion (indicated with yellow arrow) on the dissected muscle from the outer side of the right thigh (plane perpendicular to the beam).

The depth of the formed lesions (two lesions were visible after the muscle vertical dissection) was calculated to be 6 mm and 3 mm for lesion 2 and 3, respectively while 10 mm of the muscle remained unaffected (from the sonication side). Figure 18 demonstrates the dissected tissue of the rabbit muscle of the right thigh.

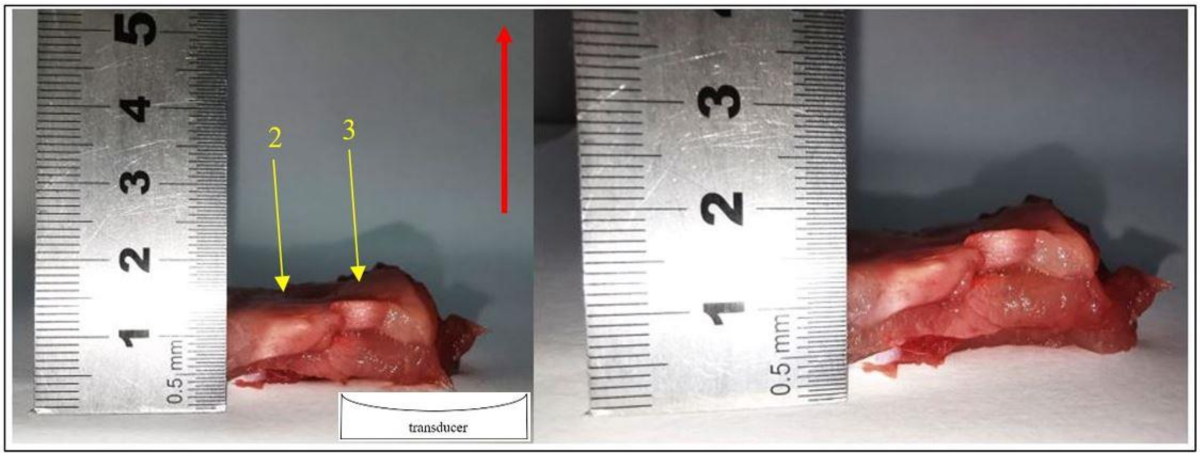

Figure 18: In situ dissection of the formed lesions of the right thigh. The yellow arrows indicate the formed lesions and the red arrow indicates the beam direction.

Three (3) lesions were observed on the right thigh after the treatment (Table 4). The diameter and length of the two lesions was measurable.

Table 4: Summary of results for the right thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	5	-
2	8	6
3	5	4

Table 5 lists the anesthesia record for the rabbit experiment.

Table 5: Anesthesia record for rabbit 2.

Animal ID	SOUNDPET_2020_2
Species	Rabbit
Sex	M
Age	Adult
Weight (Kg)	2.7
Supplier	S. Ioannou Farm (CYCU5.05)
Grant	SOUNDPET (INTEGRATED/0918/0008)
Study	CY/EXP/PR.L01/2020
Participants	Kyriakos Spanoudes
	Theocharis Drakos
	Nikolas Evripidou
Principal Investigator	Christakis Damianou
Time	**Anesthesia**
13:00	Medetomidine 1.5 ml + Ketamine 0.45 ml
13:25	Medetomidine 1 ml + Ketamine 0.4 ml
Euthanasia	13:40 1 ml T-61

Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
13:00	120	60	1	Absent	n/a
13:10	110	72	-	Absent	n/a
13:25	110	60	-	Absent	n/a
13:40	110	72	-	Absent	n/a

Rabbit 3

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	200
Acoustic power (W)	62
Sonication time (s)	5
Energy (J)	310
Cooling time (s)	60
Number of sonications	3
Step between sonications (mm)	10
Distance transducer/rabbit skin (cm)	7

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved reverse from point 1 to 3 at step movement of 10 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

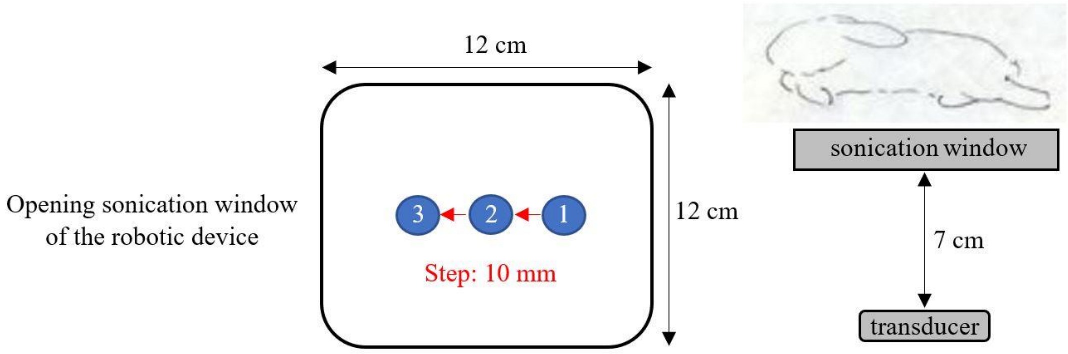

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	200
Acoustic power (W)	62
Sonication time (s)	10
Energy (J)	620
Cooling time (s)	60
Number of sonications	3
Step between sonications (mm)	10
Distance transducer/rabbit skin (cm)	7

A linear grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved reverse from point 1 to 3 at step movement of 10 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

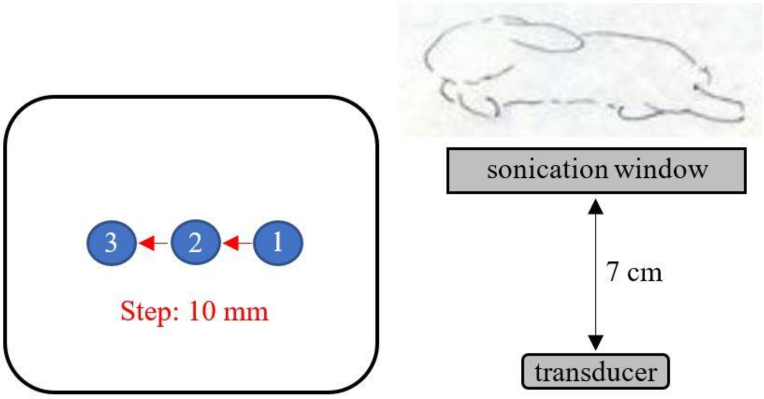

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory that was used for the rabbit thigh ablations.

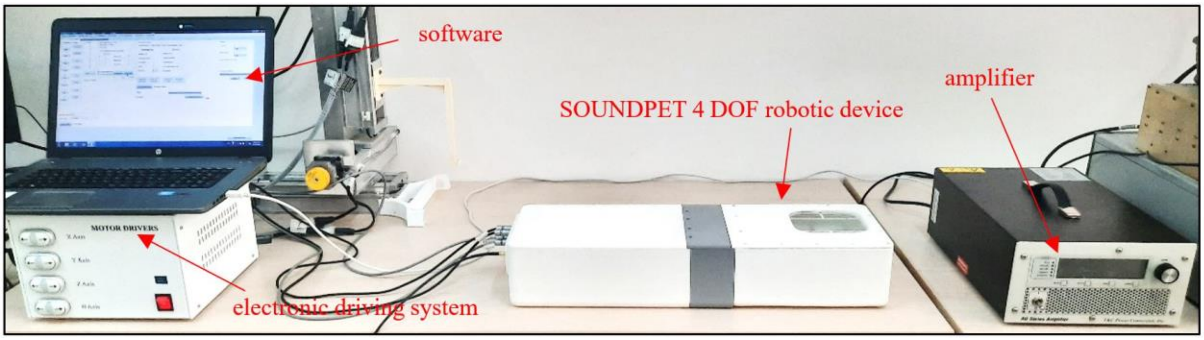

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

Figure 4: Animal positioning for the experiment.

Results

As shown in Figure 5 and Figure 6, no lesions were appeared from the side of the sonication (outer side) on the left thigh above and below the skin, respectively.

Figure 5: The outer side of the left thigh above the skin after the treatment.

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side).

Necrotic area was observed on the inner side of the left thigh both above and below the skin as shown in Figure 7 and Figure 8, respectively.

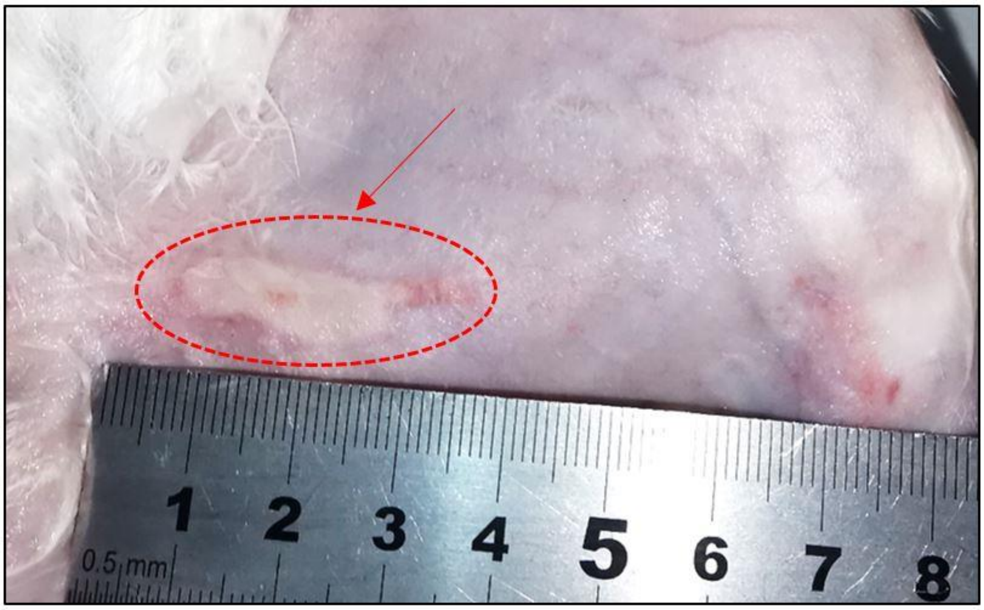

Figure 7: Macroscopic appearance of the necrosis from the inner side of the left thigh and above the skin. The red arrow indicates the necrotic area on the skin.

The formed lesions were appeared overlapped above the skin by creating a necrotic region of around 25 mm while below the skin they were discrete.

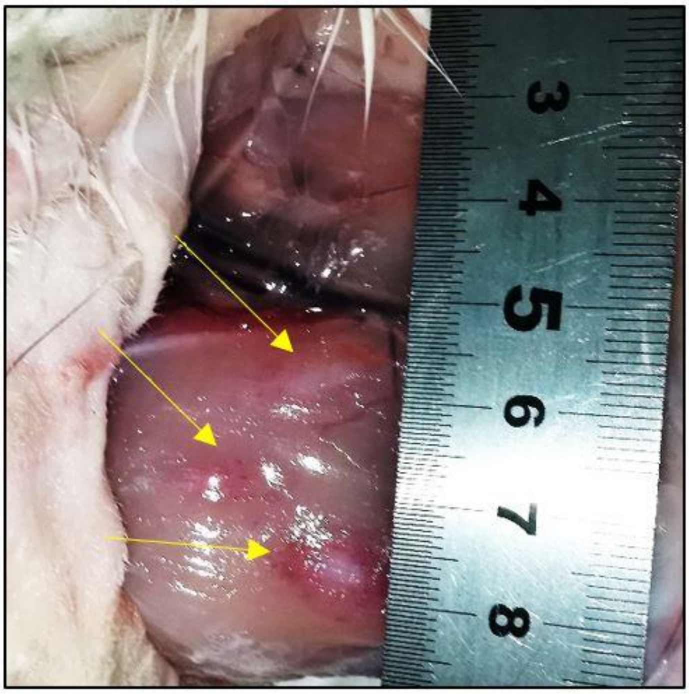

Figure 8: Macroscopic appearance of the discrete lesions on the exposed muscles from the inner side of the left thigh. The yellow arrows indicate the formed lesions after performing a linear grid of 3 sonications at step movement of 10 mm.

The muscle of the inner side of the left thigh was dissected and three (3) discrete lesions were appeared as shown in Figure 9.

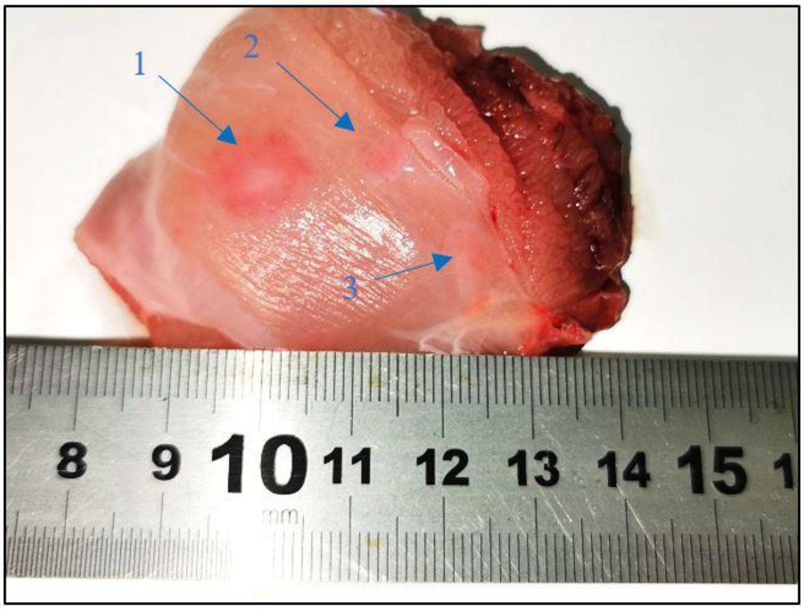

Figure 9: Macroscopic appearance of the formed lesions (indicated with blue arrows) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The depth of the formed lesions (two lesions were visible after the muscle vertical dissection) was calculated to be 8 mm and 4 mm for lesion 1 and 2, respectively while 7 mm of the muscle remained unaffected. Figure 10 and Figure 11 demonstrates the dissection of the lesion 1 and 2 that were created on the left thigh, respectively.

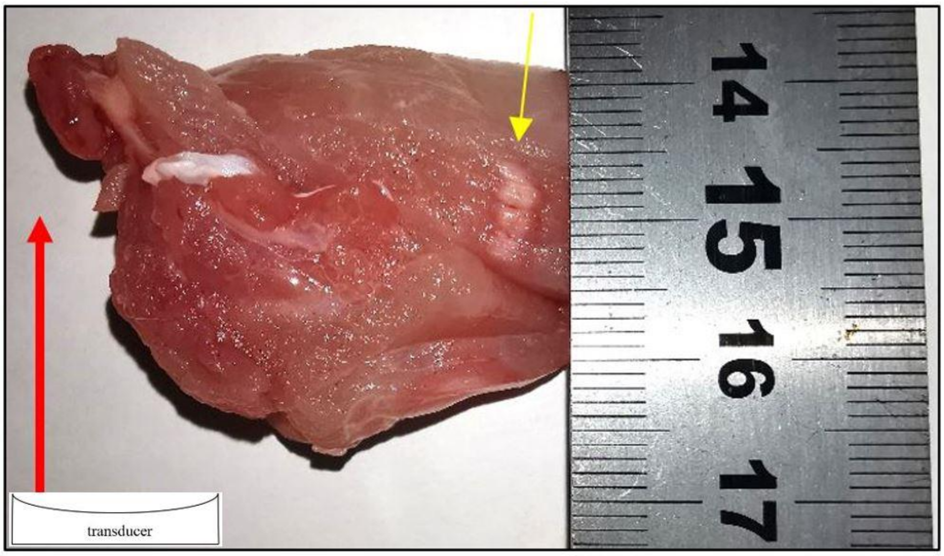

Figure 10: In situ dissection of the formed lesion 1 of the left thigh. The yellow arrow indicates the formed lesion and the red arrow indicates the beam direction.

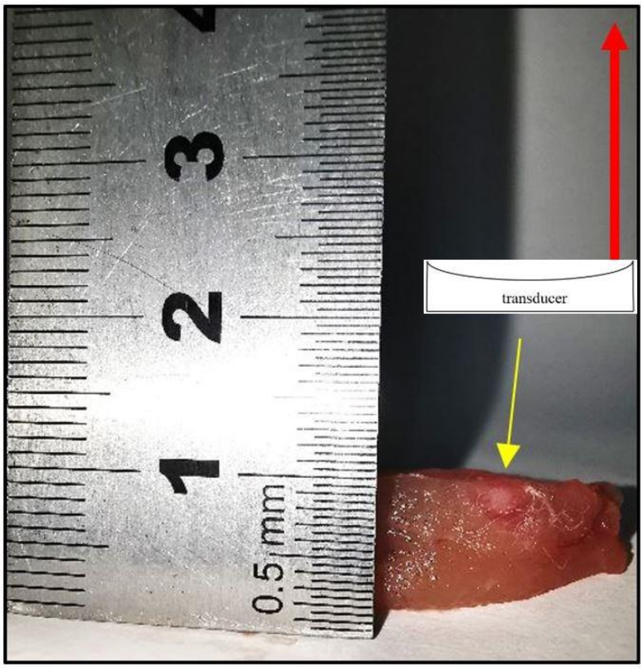

Figure 11: In situ dissection of the formed lesion 2 of the left thigh. The yellow arrow indicates the formed lesion and the red arrow indicates the beam direction.

Three (3) lesions were observed on the left thigh after the treatment (Table 3). The diameter and length of the two lesions was measurable.

Table 3: Summary of results for the left thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	6	8
2	5	4
3	5	-

As shown in Figure 12 and Figure 13, no lesions were appeared on the outer side of the right thigh above and below the skin, respectively.

Figure 12: The outer side of the right thigh above the skin after the treatment.

Figure 13: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed.

Necrotic area was observed on the inner side of the right thigh both above and below the skin as shown in Figure 14 and Figure 15, respectively.

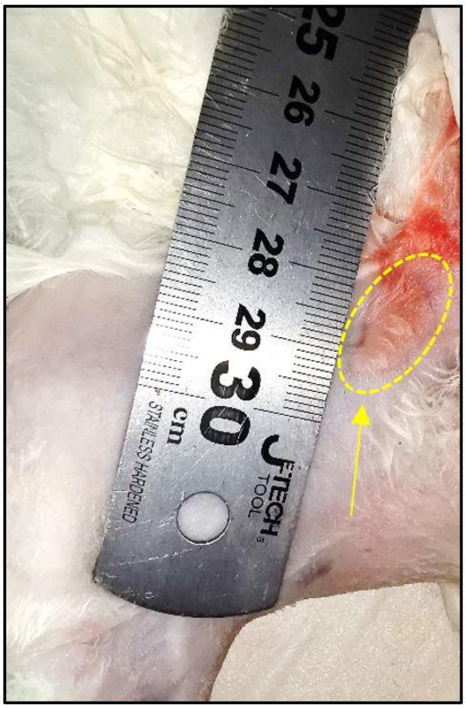

Figure 14: Macroscopic appearance of the necrosis from the inner side of the right thigh and above the skin. The yellow arrow indicates the necrotic area on the skin.

The formed lesions were appeared overlapped above the skin by creating a necrotic region of around 15 mm while below the skin they were discrete.

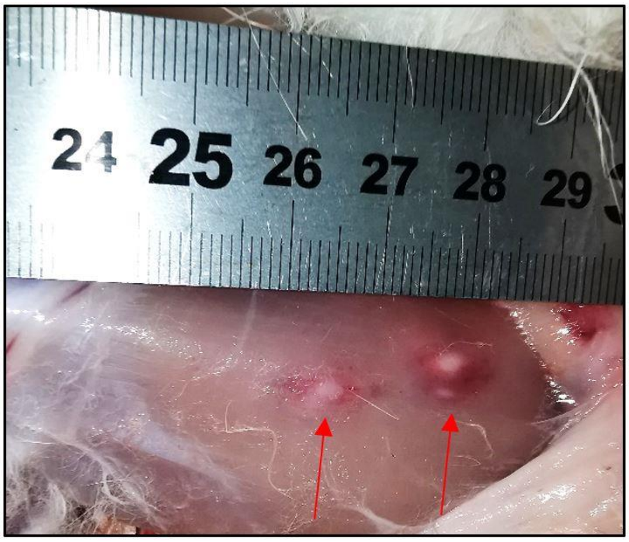

Figure 15: Macroscopic appearance of the discrete lesions on the exposed muscles from the inner side of the right thigh. The red arrows indicate the formed lesions after performing a linear grid of 3 sonications at step movement of 10 mm.

The muscle of the inner side of the right thigh was dissected and two (2) discrete lesions were appeared as shown in Figure 16.

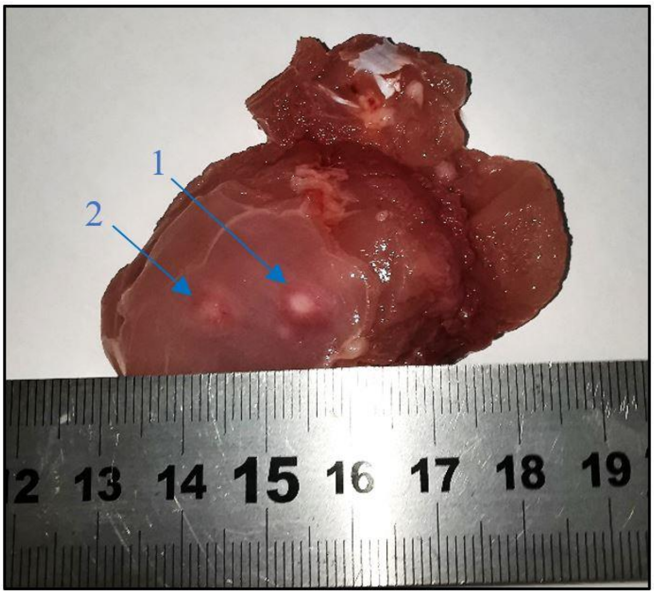

Figure 16: Macroscopic appearance of the formed lesions (indicated with blue arrows) on the dissected muscle of the inner side of the right thigh (plane perpendicular to the beam).

The depth of the formed lesions (two lesions were visible after the muscle vertical dissection) was calculated to be 17 mm and 4 mm for lesion 1 and 2, respectively. Figure 17 and Figure 18 demonstrates the dissection of the lesion 1 and 2 that were created on the right thigh, respectively.

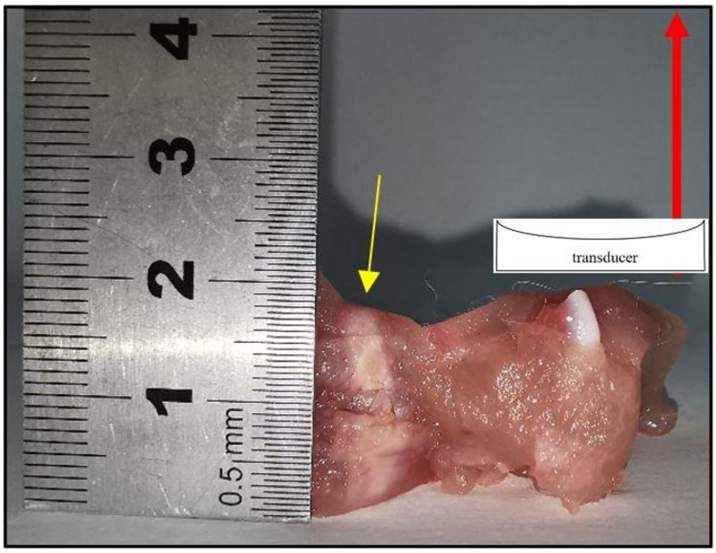

Figure 17: In situ dissection of the formed lesion 1 of the right thigh. The yellow arrow indicates the formed lesion and the red arrow indicates the beam direction.

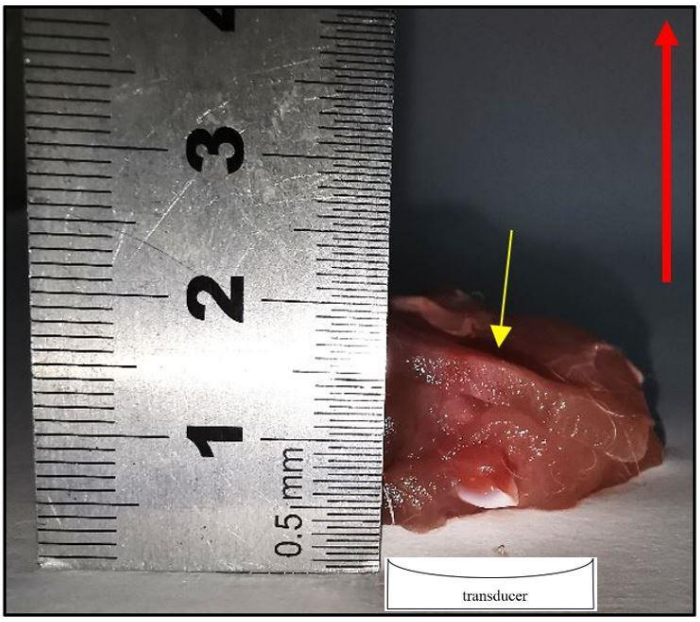

Figure 18: In situ dissection of the formed lesion 2 of the right thigh. The yellow arrow indicates the formed lesion and the red arrow indicates the beam direction.

Two (2) lesions were observed on the right thigh after the treatment (Table 4). The diameter and length of the two lesions was measurable.

Table 4: Summary of results for the right thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	4	17
2	3	4

Table 5 lists the anesthesia record for the rabbit experiment.

Table 5: Anesthesia record for rabbit 3.

Animal ID	SOUNDPET_2020_3				
Species	Rabbit				
Sex	M				
Age	Adult				
Weight (Kg)	2.20				
Supplier	S. Ioannou Farm (CYCU5.05)				
Grant	SOUNDPET (INTEGRATED/0918/0008)				
Study	CY/EXP/PR.L01/2020				
Participants	Kyriakos Spanoudes				
	Theocharis Drakos				
	Nikolas Evripidou				
Principal Investigator	Christakis Damianou				
Time	**Anesthesia**				
09:45	Medetomidine 1.2 ml + Ketamine 0.3 ml				
10:15	Medetomidine 0.6 ml + Ketamine 0.15 ml				
Euthanasia	10:30 1 ml T-61				
Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
10:00	100	40	-	Absent	n/a
10:10	110	42	-	Absent	n/a
10:20	100	40	-	Absent	n/a

Rabbit 4

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	200
Acoustic power (W)	62
Sonication time (s)	10
Energy (J)	620
Cooling time (s)	60
Number of sonications	3
Step between sonications (mm)	10
Distance transducer/rabbit skin (cm)	7

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved reverse from point 1 to 3 at step movement of 10 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

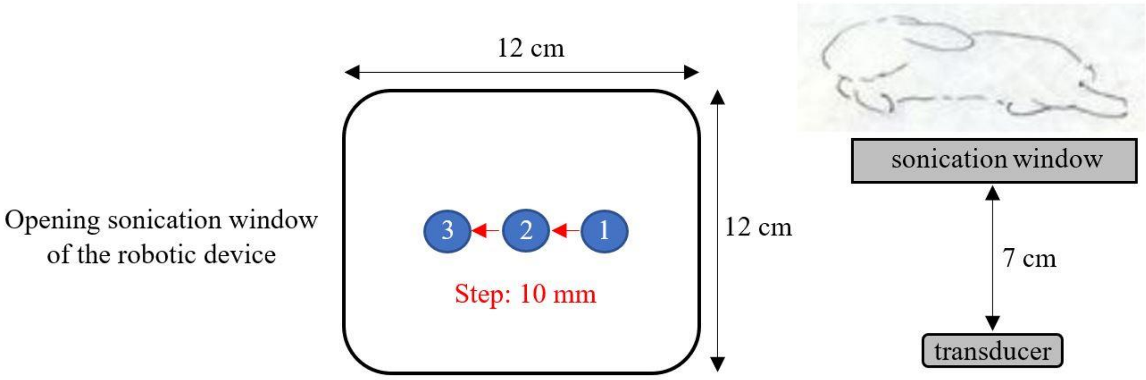

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	250
Acoustic power (W)	77.5
Sonication time (s)	5
Energy (J)	387.5
Cooling time (s)	60
Number of sonications	3
Step between sonications (mm)	10
Distance transducer/rabbit skin (cm)	7

A linear grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved reverse from point 1 to 3 at step movement of 10 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

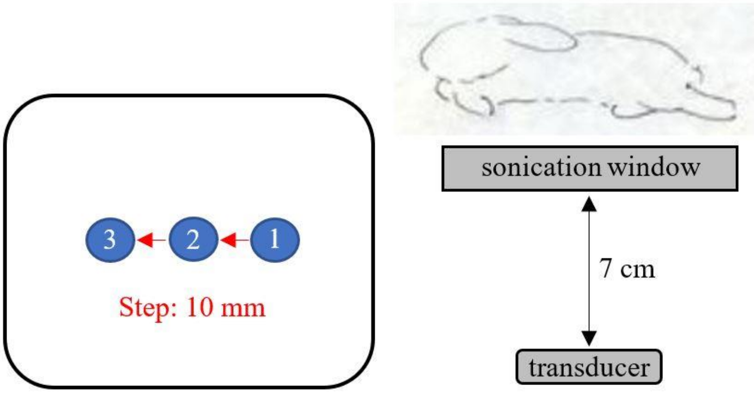

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory that was used for the rabbit thigh ablations.

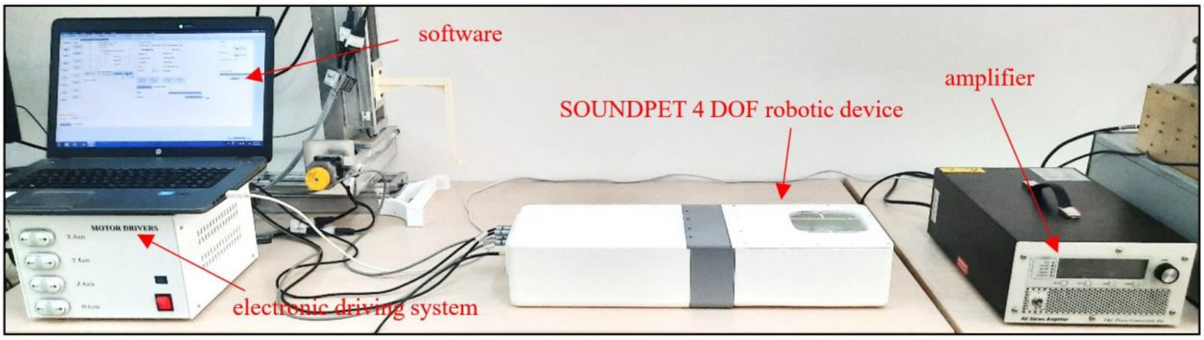

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

Figure 4: Animal positioning for the experiment.

Results

As shown in Figure 5 and Figure 6, no lesions were appeared from the side of the sonication (outer side) on the left thigh above and below the skin, respectively.

Figure 5: The outer side of the left thigh above the skin after the treatment.

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side).

Discrete lesions were observed on the inner side of the left thigh both above and below the skin as shown in Figure 7 and Figure 8, respectively.

Figure 7: Macroscopic appearance of the formed lesions from the inner side of the left thigh and above the skin. The red arrows indicate the discrete lesions on the skin.

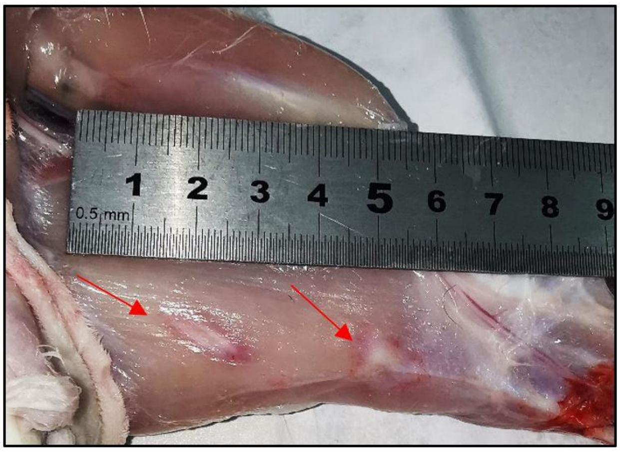

Figure 8: Macroscopic appearance of the discrete lesions on the exposed muscles from the inner side of the left thigh. The red arrows indicate the formed lesions after performing a linear grid of 3 sonications at step movement of 10 mm.

The muscle of the inner side of the left thigh was dissected and two (2) discrete lesions were appeared as shown in Figure 9.

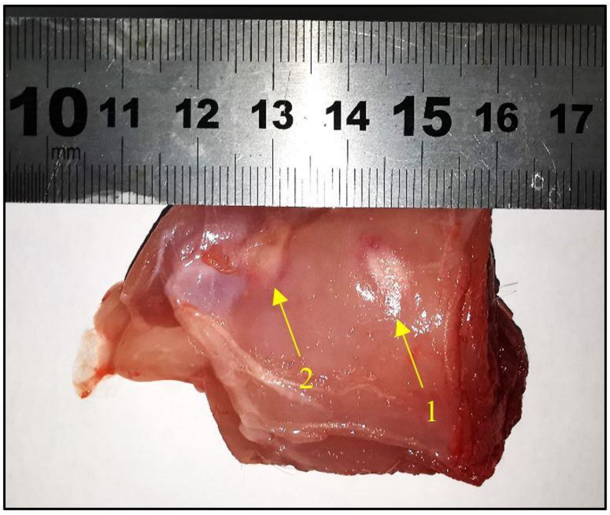

Figure 9: Macroscopic appearance of the formed lesions (indicated with yellow arrows) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The depth of the formed lesions (two lesions were visible after the muscle vertical dissection) was calculated to be 10 mm and 6 mm for lesion 1 and 2, respectively. Figure 10 and Figure 11 demonstrates the dissection of the lesion 1 and 2 that were created on the left thigh, respectively.

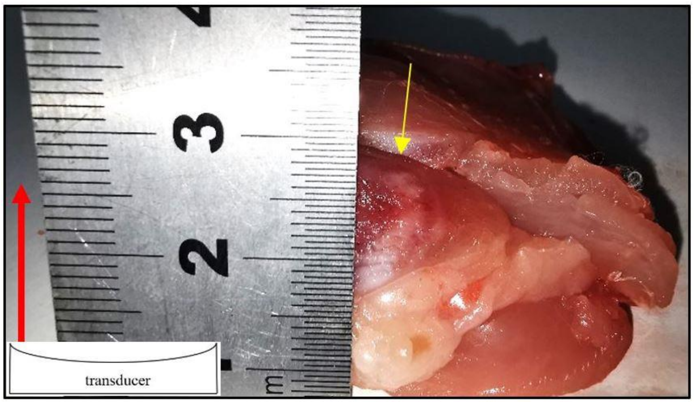

Figure 10: In situ dissection of the formed lesion 1 of the left thigh. The yellow arrow indicates the formed lesion and the red arrow indicates the beam direction.

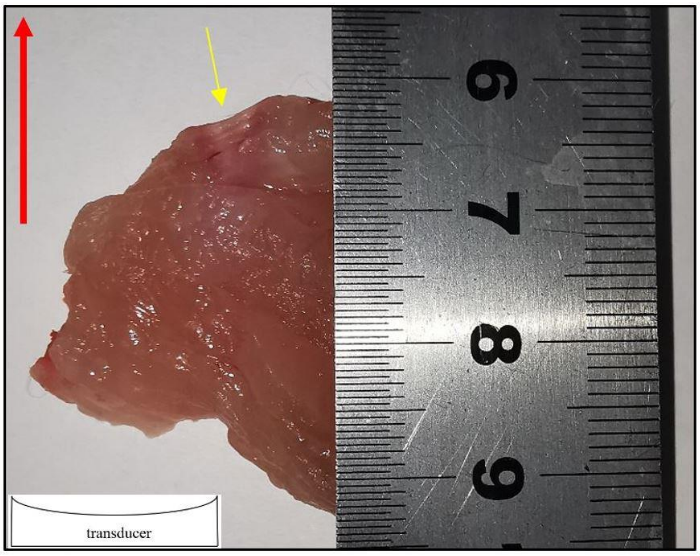

Figure 11: In situ dissection of the formed lesion 2 of the left thigh. The yellow arrow indicates the formed lesion and the red arrow indicates the beam direction.

Two (2) lesions were observed on the left thigh after the treatment (Table 3). The diameter and length of the two lesions was measurable.

Table 3: Summary of results for the left thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	7	10
2	5	6

As shown in Figure 12 and Figure 13, no lesions were appeared on the outer side of the right thigh above and below the skin, respectively.

Figure 12: The outer side of the right thigh above the skin after the treatment.

Figure 13: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed.

Discrete lesions were observed on the inner side of the right thigh both above and below the skin as shown in Figure 14 and Figure 15, respectively.

Figure 14: Macroscopic appearance of the formed lesions from the inner side of the right thigh and above the skin. The red arrows indicate the lesions on the skin.

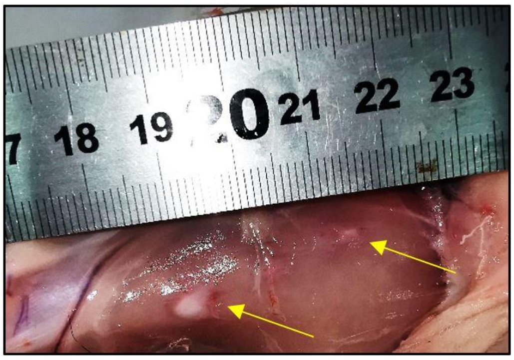

Figure 15: Macroscopic appearance of the discrete lesions on the exposed muscles from the inner side of the right thigh. The yellow arrows indicate the formed lesions after performing a linear grid of 3 sonications at step movement of 10 mm.

The muscle of the inner side of the right thigh was dissected and three (3) discrete lesions were appeared as shown in Figure 16.

Figure 16: Macroscopic appearance of the formed lesions (indicated with red arrows) on the dissected muscle of the inner side of the right thigh (plane perpendicular to the beam).

The depth of the formed lesions (all lesions were visible after the muscle vertical dissection) was calculated to be 3 mm, 1 mm, and 2 mm for lesion 1, 2, and 3, respectively. Figure 17, Figure 18, and Figure 19 demonstrates the dissection of the lesion 1, 2, and 3 that were created on the right thigh, respectively.

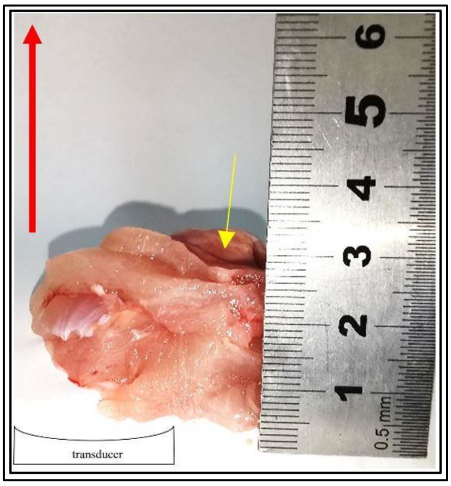

Figure 17: In situ dissection of the formed lesion 1 of the right thigh. The yellow arrow indicates the formed lesion and the red arrow indicates the beam direction.

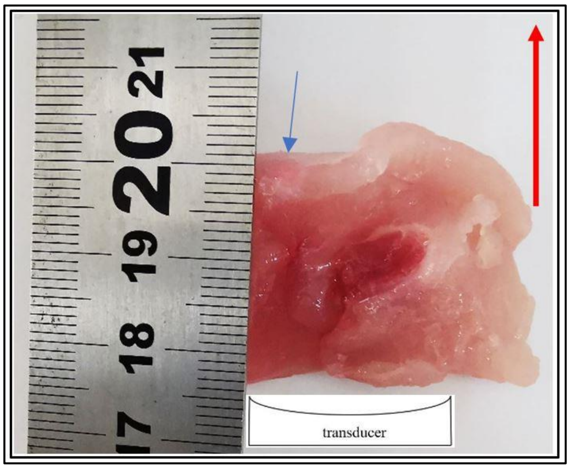

Figure 18: In situ dissection of the formed lesion 2 of the right thigh. The blue arrow indicates the formed lesion and the red arrow indicates the beam direction.

Figure 19: In situ dissection of the formed lesion 3 of the right thigh. The blue arrow indicates the formed lesion and the red arrow indicates the beam direction.

Three (3) lesions were observed on the right thigh after the treatment (Table 4). The diameter and length of the three lesions was measurable.

Table 4: Summary of results for the right thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	6	3
2	6	1
3	5	2

Table 5 lists the anesthesia record for the rabbit experiment.

Table 5: Anesthesia record for rabbit 4.

Animal ID	SOUNDPET_2020_4
Species	Rabbit
Sex	M
Age	Adult
Weight (Kg)	2.20
Supplier	S. Ioannou Farm (CYCU5.05)
Grant	SOUNDPET (INTEGRATED/0918/0008)
Study	CY/EXP/PR.L01/2020
Participants	Kyriakos Spanoudes
	Theocharis Drakos
	Nikolas Evripidou
Principal Investigator	Christakis Damianou

Time	**Anesthesia**
11:35	Medetomidine 1.2 ml + Ketamine 0.3 ml
11:55	Medetomidine 0.7 ml
Euthanasia	12:30 1 ml T-61

Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
11:35	110	42	-	Absent	n/a
11:45	100	46	-	Absent	n/a
12:00	100	42	-	Absent	n/a

Rabbit 5

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	200
Acoustic power (W)	62
Sonication time (s)	5
Energy (J)	310
Cooling time (s)	60
Number of sonications	3
Step between sonications (mm)	10
Distance transducer/rabbit skin (cm)	7

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved reverse from point 1 to 3 at step movement of 10 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

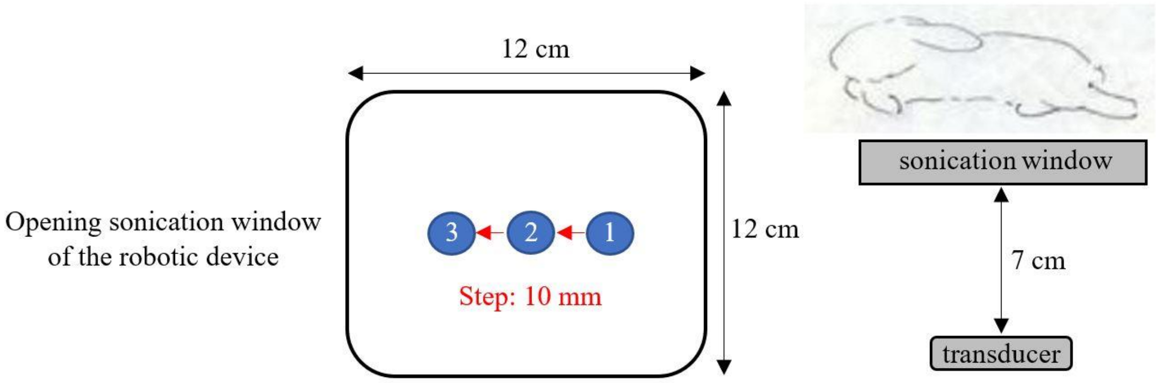

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	200
Acoustic power (W)	62
Sonication time (s)	10
Energy (J)	620
Cooling time (s)	60
Number of sonications	3
Step between sonications (mm)	10
Distance transducer/rabbit skin (cm)	7

A linear grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved reverse from point 1 to 3 at step movement of 10 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

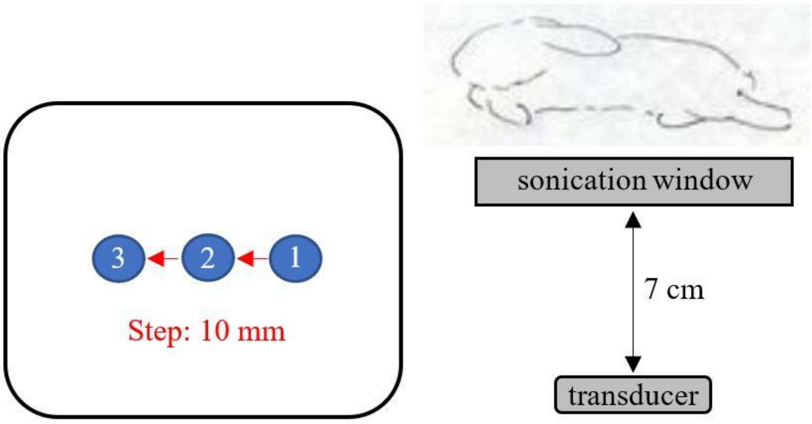

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory that was used for the rabbit thigh ablations.

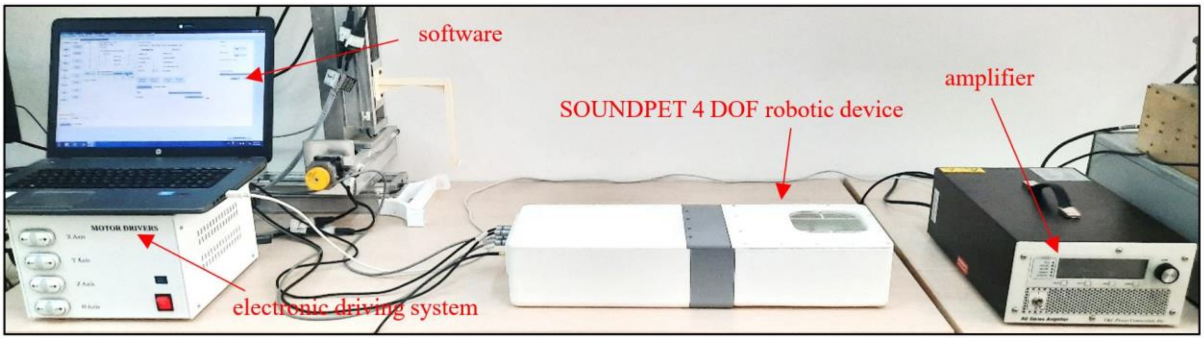

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

Figure 4: Animal positioning for the experiment.

Results

As shown in Figure 5 and Figure 6, no lesions were appeared from the side of the sonication (outer side) on the left thigh above and below the skin, respectively.

Figure 5: The outer side of the left thigh above the skin after the treatment.

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side).

Two (2) overlapping lesions were observed on the inner side of the left thigh above the skin but below the skin no lesion was observed as shown in Figure 7 and Figure 8, respectively.

Figure 7: Macroscopic appearance of the formed lesions from the inner side of the left thigh and above the skin. The red arrows indicate the overlapping lesions on the skin.

Figure 8: Macroscopic appearance of the exposed muscle from the inner side of the left thigh. No lesions were formed after performing a linear grid of 3 sonications at step movement of 10 mm.

The muscle of the left thigh (from the inner side) was dissected and no lesions were appeared as shown in Figure 9.

Figure 9: Macroscopic appearance of the dissected muscle (from the inner side) of the left thigh (plane perpendicular to the beam).

As shown in Figure 10, no lesions were appeared on the outer side of the right thigh above the skin. No lesions were also observed on the outer side of the right thigh below the skin.

Figure 10: The outer side of the right thigh above the skin after the treatment.

The lesions were observed to be overlapped on the inner side of the right thigh above the skin and discrete below the skin as shown in Figure 11 and Figure 12, respectively.

Figure 11: Macroscopic appearance of the formed lesions from the inner side of the right thigh and above the skin. The red arrow indicates the area of the formed lesions on the skin.

Figure 12: Macroscopic appearance of the discrete lesions on the exposed muscles from the inner side of the right thigh. The red arrows indicate the formed lesions after performing a linear grid of 3 sonications at step movement of 10 mm.

The muscle of the inner side of the right thigh was dissected and three (3) discrete lesions were appeared as shown in Figure 13.

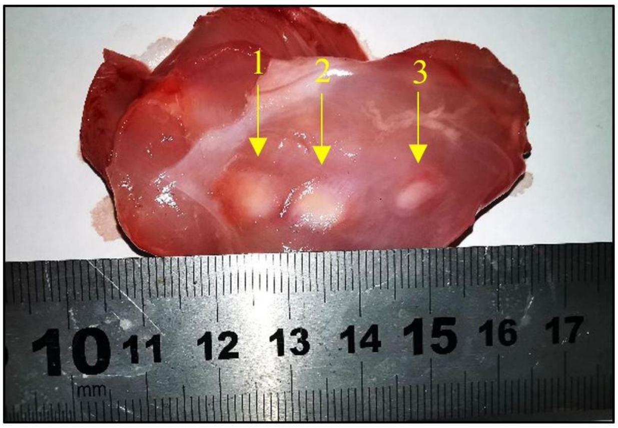

Figure 13: Macroscopic appearance of the formed lesions (indicated with yellow arrows) on the dissected muscle of the inner side of the right thigh (plane perpendicular to the beam).

The depth of the formed lesions (all lesions were visible after the muscle vertical dissection) was calculated to be 3 mm, 2 mm, and 2 mm for lesion 1, 2, and 3, respectively. Figure 14 demonstrates the dissection of the lesion 1, 2, and 3 that were created on the right thigh.

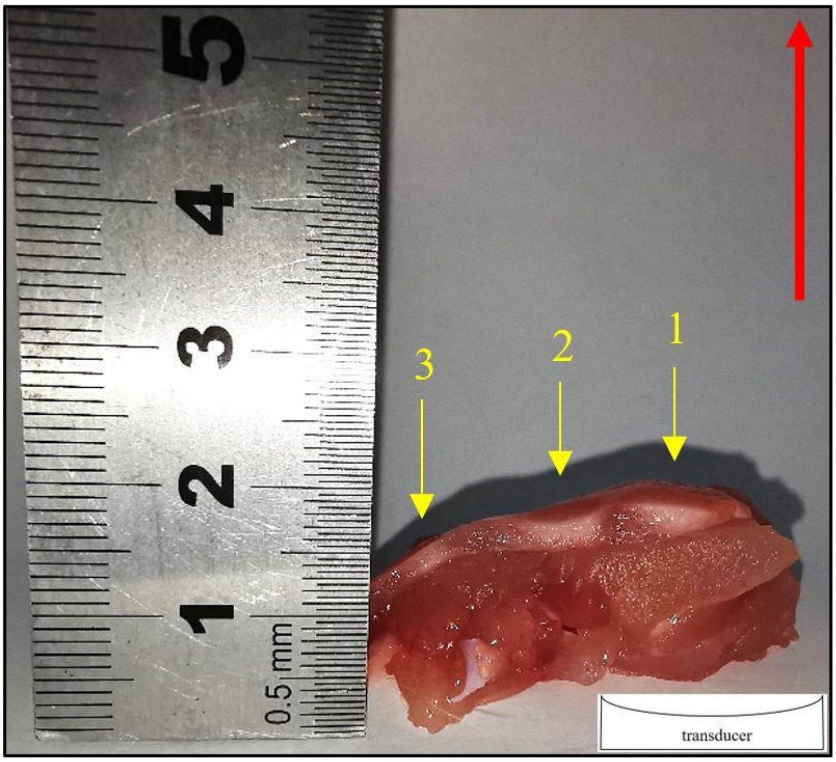

Figure 14: In situ dissection of the formed lesions 1, 2, and 3 of the right thigh. The yellow arrows indicate the formed lesion and the red arrow indicates the beam direction.

Three (3) lesions were observed on the right thigh after the treatment (Table 3). The diameter and length of the three lesions was measurable.

Table 3: Summary of results for the right thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	8	3
2	8	2
3	7	2

Table 4 lists the anesthesia record for the rabbit experiment.

Table 4: Anesthesia record for rabbit 5.

Animal ID	SOUNDPET_2020_5
Species	Rabbit
Sex	M
Age	Adult
Weight (Kg)	1.9
Supplier	S. Ioannou Farm (CYCU5.05)
Grant	SOUNDPET (INTEGRATED/0918/0008)
Study	CY/EXP/PR.L01/2020
Participants	Kyriakos Spanoudes
	Theocharis Drakos
	Nikolas Evripidou
Principal Investigator	Christakis Damianou
Time	**Anesthesia**
09:45	Medetomidine 1.1 ml + Ketamine 0.2 ml
10:00	Medetomidine 0.2 ml
10:10	Medetomidine 0.12 ml + Ketamine 0.1 ml
Euthanasia	10:30 1 ml T-61

Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
09:50	110	60	0	Absent	n/a
10:00	100	52	1	Absent	n/a
10:10	110	56	0	Absent	n/a
10:20	110	52	0	Absent	n/a

Rabbit 6

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	250
Acoustic power (W)	77.5
Sonication time (s)	5
Energy (J)	387.5
Cooling time (s)	60
Number of sonications	3
Step between sonications (mm)	10
Distance transducer/rabbit skin (cm)	7

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved reverse from point 1 to 3 at step movement of 10 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

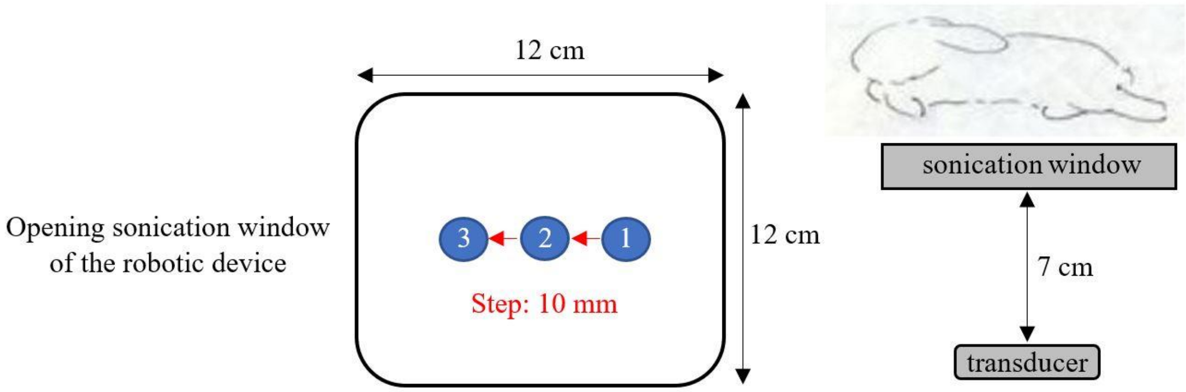

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	250
Acoustic power (W)	77.5
Sonication time (s)	10
Energy (J)	775
Cooling time (s)	60
Number of sonications	3
Step between sonications (mm)	10
Distance transducer/rabbit skin (cm)	7

A linear grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved reverse from point 1 to 3 at step movement of 10 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

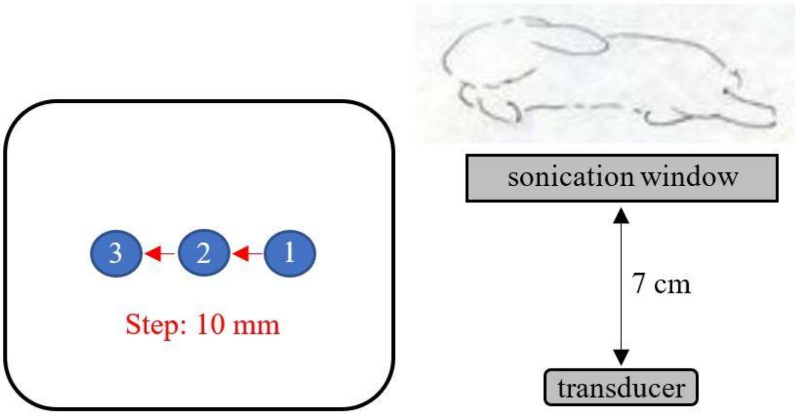

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory that was used for the rabbit thigh ablations.

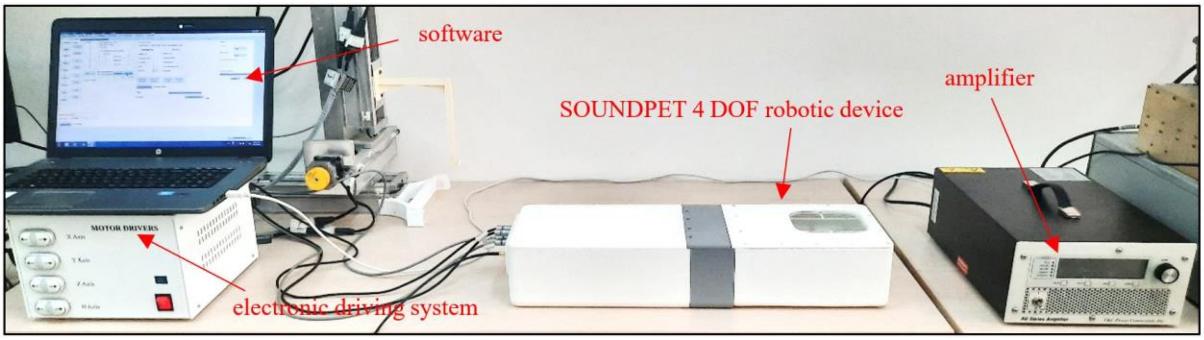

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

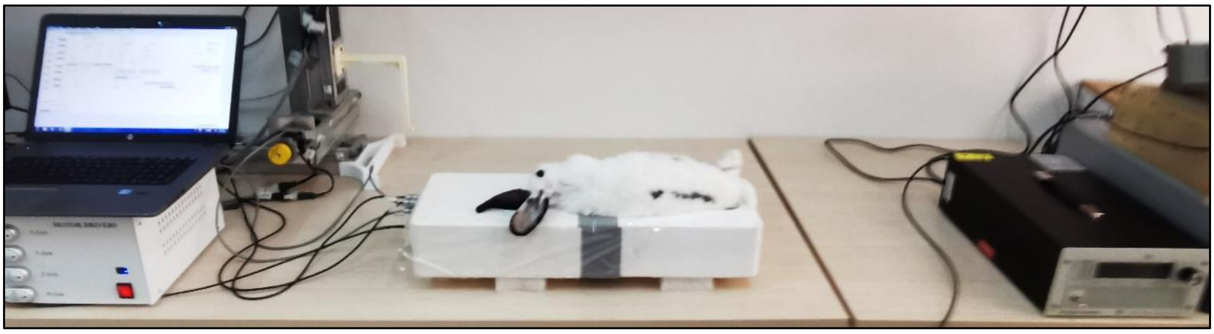

Figure 4: Animal positioning for the experiment.

Results

As shown in Figure 5, three (3) discrete lesions were appeared from the side of the sonication (outer side) on the left thigh above the skin while they were not visible below the skin as shown in Figure 6.

Figure 5: The outer side of the left thigh above the skin after the treatment.

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side).

Three (3) discrete lesions were observed on the inner side of the left thigh above the skin but below the skin no lesion was observed as shown in Figure 7 and Figure 8, respectively.

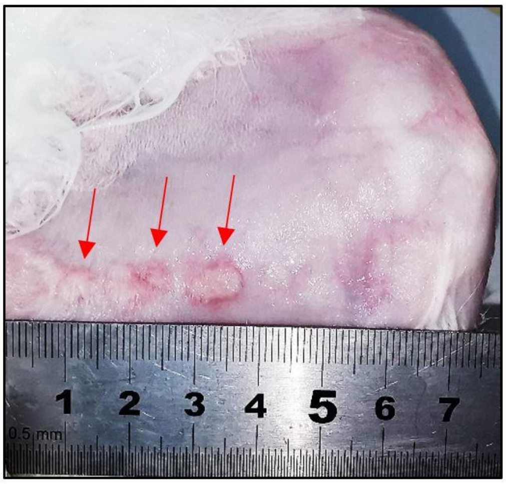

Figure 7: Macroscopic appearance of the formed lesions from the inner side of the left thigh and above the skin. The red arrows indicate the discrete lesions on the skin.

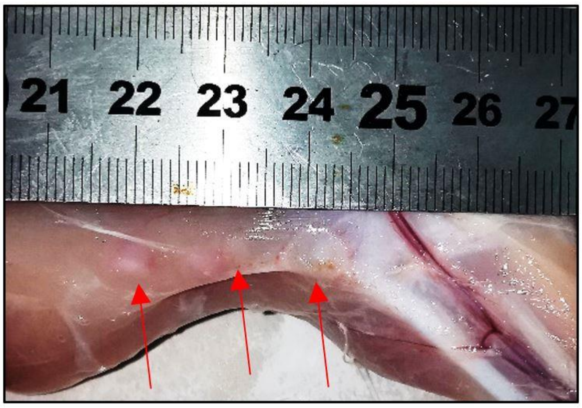

Figure 8: Macroscopic appearance of the exposed muscle from the inner side of the left thigh. No lesions were formed after performing a linear grid of 3 sonications at step movement of 10 mm. The red arrows indicate the discrete lesions on the exposed muscle.

The muscle of the inner side of the left thigh was dissected and three (3) discrete lesions were appeared as shown in Figure 9.

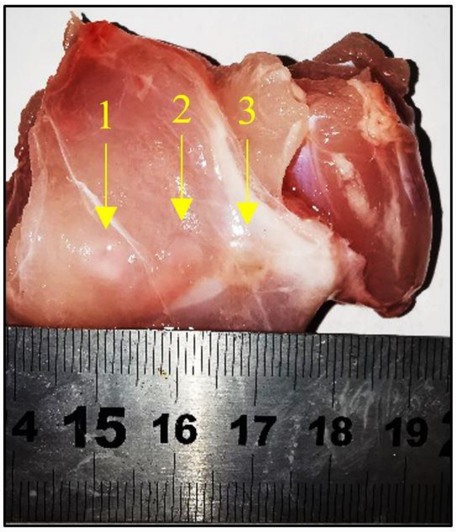

Figure 9: Macroscopic appearance of the formed lesions (indicated with yellow arrows) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The depth of the formed lesions (two lesions were visible after the muscle vertical dissection) was calculated to be 6 mm and 2 mm for the lesion 1 and 2, respectively. Figure 10 and Figure 11 demonstrates the dissection of the lesion 1 and 2 that were created on the left thigh.

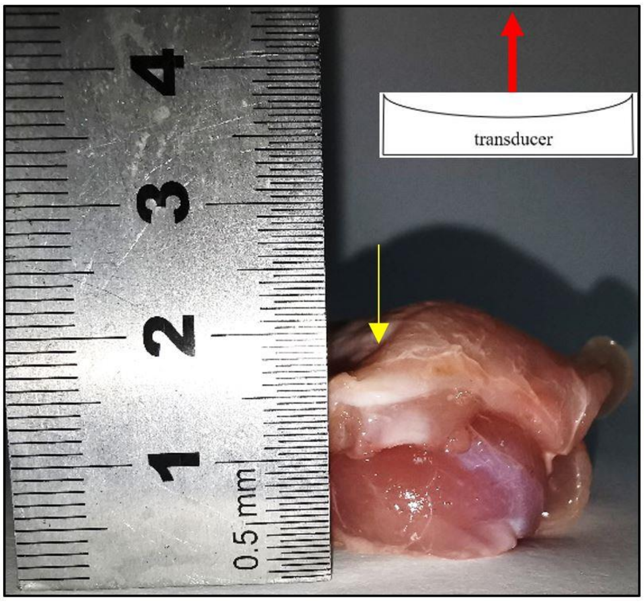

Figure 10: In situ dissection of the formed lesion 1 of the left thigh. The yellow arrow indicates the formed lesion and the red arrow indicates the beam direction.

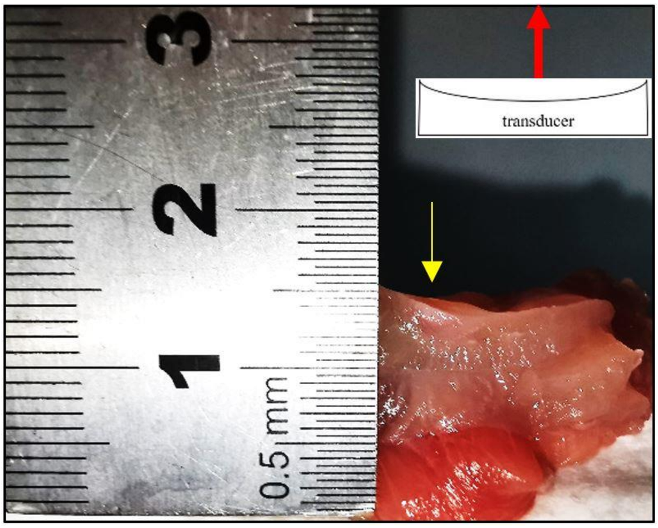

Figure 11: In situ dissection of the formed lesion 2 of the left thigh. The yellow arrow indicates the formed lesion and the red arrow indicates the beam direction.

Three (3) lesions were observed on the right thigh after the treatment (Table 3). The diameter of all lesions was measurable while the length of the two lesions was measurable.

Table 3: Summary of results for the right thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	5	6
2	5	2
3	5	-

As shown in Figure 12 and Figure 13, no lesions were appeared on the outer side of the right thigh above and below the skin, respectively.

Figure 12: The outer side of the right thigh above the skin after the treatment.

Figure 13: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed.

The lesions were observed to be overlapped on the inner side of the right thigh above the skin and discrete below the skin as shown in Figure 14 and Figure 15, respectively.

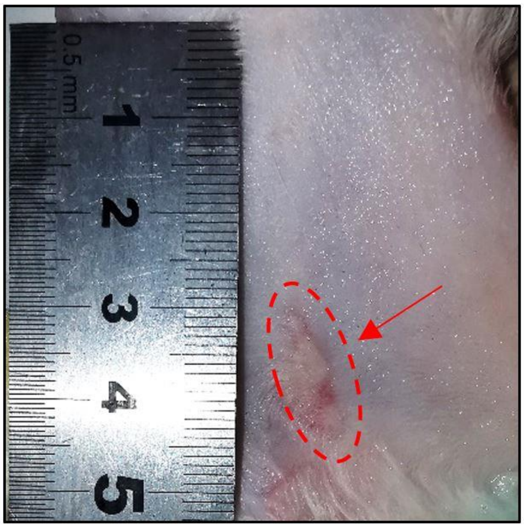

Figure 14: Macroscopic appearance of the formed lesions from the inner side of the right thigh and above the skin. The red arrow indicates the area of the formed lesions on the skin.

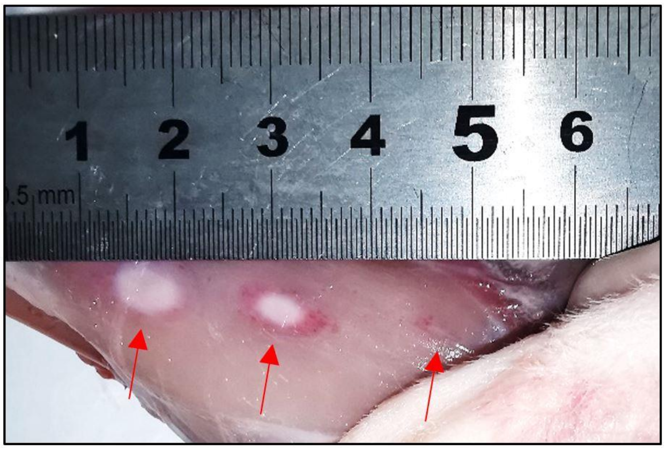

Figure 15: Macroscopic appearance of the discrete lesions on the exposed muscles from the inner side of the right thigh. The red arrows indicate the formed lesions after performing a linear grid of 3 sonications at step movement of 10 mm.

The muscle of the inner side of the right thigh was dissected and two (2) discrete lesions were appeared as shown in Figure 16. The third lesion was very close to the bone and it was impossible to be dissected.

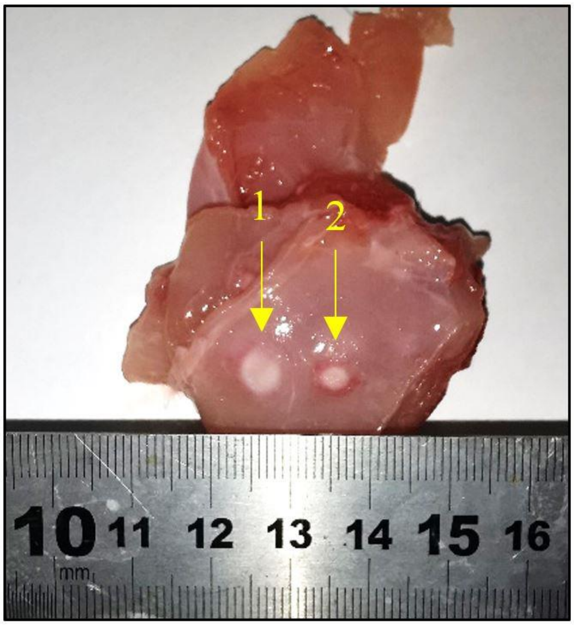

Figure 16: Macroscopic appearance of the formed lesions (indicated with yellow arrows) on the dissected muscle of the inner side of the right thigh (plane perpendicular to the beam).

The depth of the formed lesions (two lesions were visible after the muscle vertical dissection) was calculated to be 11 mm and 8 mm for lesion 1 and 2, respectively. Figure 17 and Figure 18 demonstrates the dissection of the lesion 1 and 2 that were created on the right thigh, respectively.

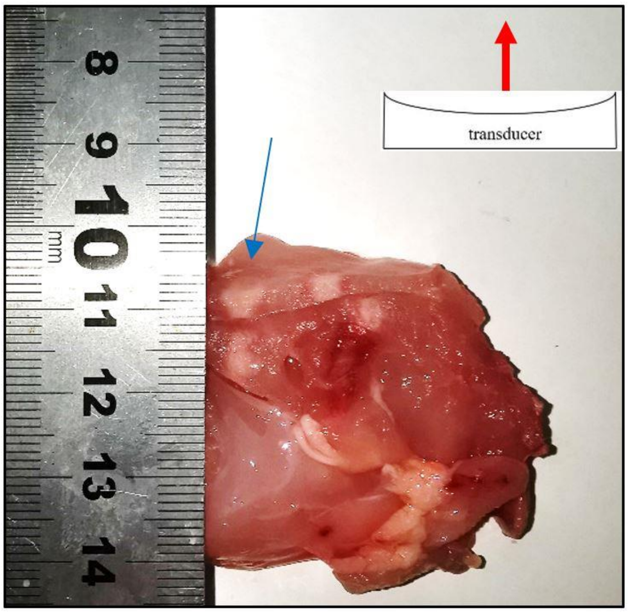

Figure 17: In situ dissection of the formed lesion 1 of the right thigh. The blue arrow indicates the formed lesion and the red arrow indicates the beam direction.

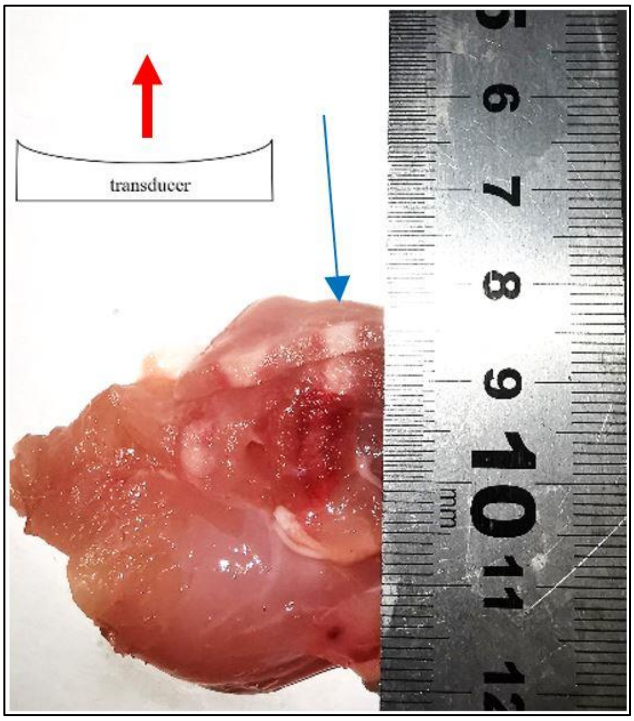

Figure 18: In situ dissection of the formed lesion 2 of the right thigh. The blue arrow indicates the formed lesion and the red arrow indicates the beam direction.

Two (2) lesions were observed on the right thigh after the treatment (Table 4). The diameter and length of the two lesions was measurable.

Table 4: Summary of results for the right thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	5	11
2	4	8
3	-	-

Table 5 lists the anesthesia record for the rabbit experiment.

Table 5: Anesthesia record for rabbit 6.

Animal ID	SOUNDPET_2020_6
Species	Rabbit
Sex	M
Age	Adult
Weight (Kg)	2.15
Supplier	S. Ioannou Farm (CYCU5.05)
Grant	SOUNDPET (INTEGRATED/0918/0008)
Study	CY/EXP/PR.L01/2020
Participants	Kyriakos Spanoudes
	Theocharis Drakos
	Nikolas Evripidou
Principal Investigator	Christakis Damianou
Time	**Anesthesia**
10:45	Medetomidine 1.2 ml + Ketamine 0.3 ml
11:00	Medetomidine 0.3 ml
11:15	Medetomidine 0.8 ml + Ketamine 0.1 ml
Euthanasia	11:40 1 ml T-61

Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
10:50	100	70	0	Absent	n/a
11:00	110	62	0	Absent	n/a
11:10	100	62	0	Absent	n/a
11:25	100	66	3	Absent	n/a

Rabbit 7

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	200
Acoustic power (W)	62
Sonication time (s)	10
Energy (J)	620
Cooling time (s)	90
Number of sonications	9
Step between sonications (mm)	5
Distance transducer/rabbit skin (cm)	7

A 3 x 3 grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 9 at step movement of 5 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

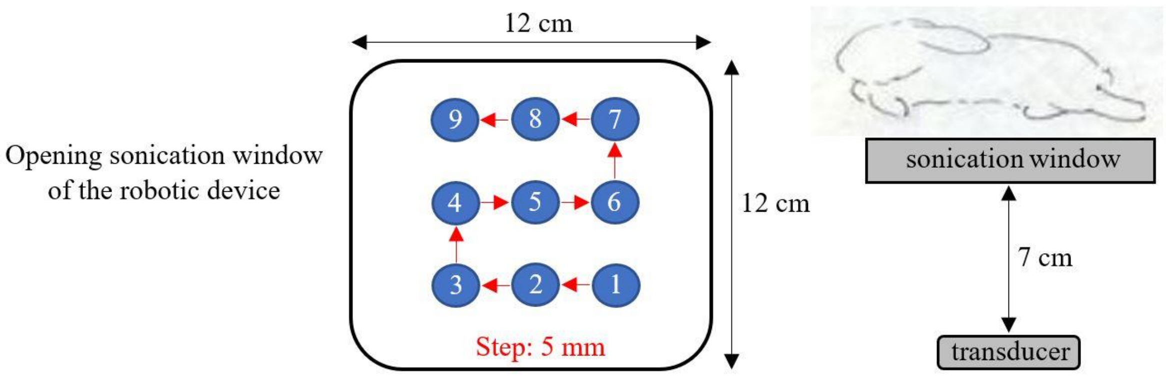

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	200
Acoustic power (W)	62
Sonication time (s)	5
Energy (J)	310
Cooling time (s)	90
Number of sonications	9
Step between sonications (mm)	5
Distance transducer/rabbit skin (cm)	7

A 3 x 3 grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved from point 1 to 9 at step movement of 5 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

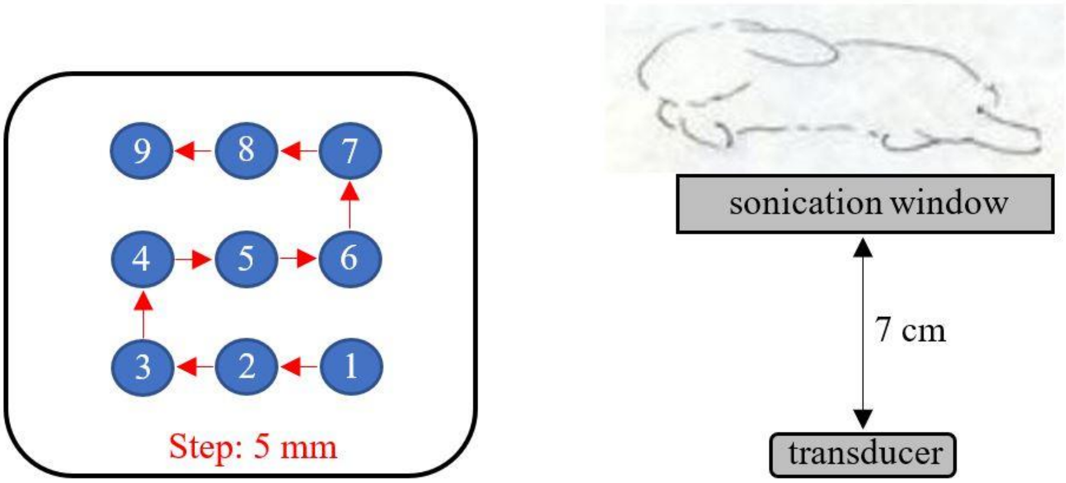

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory that was used for the rabbit thigh ablations.

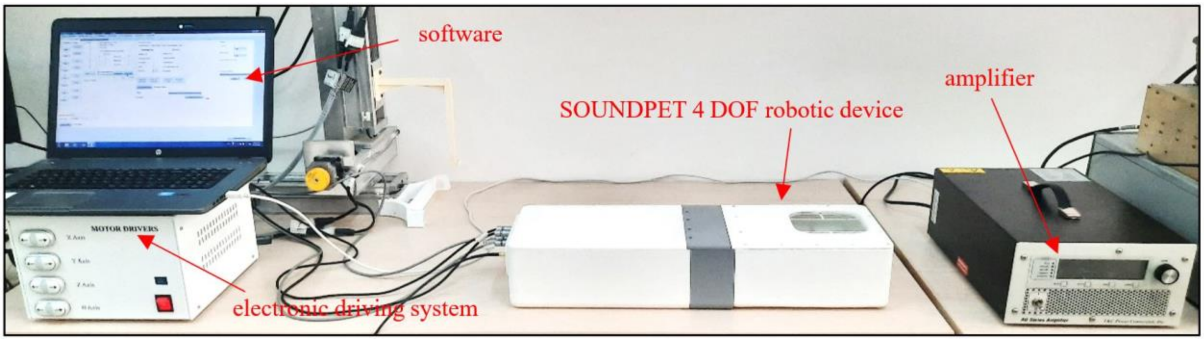

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

Figure 4: Animal positioning for the experiment.

Results

No lesions were appeared from the side of the sonication (outer side) on the left thigh above and below the skin as shown in Figure 5 and Figure 6, respectively.

Figure 5: The outer side of the left thigh above the skin after the treatment.

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side).

Overlapping lesions were appeared on the inner side of the left thigh above and below the skin as shown in Figure 7 and Figure 8, respectively.

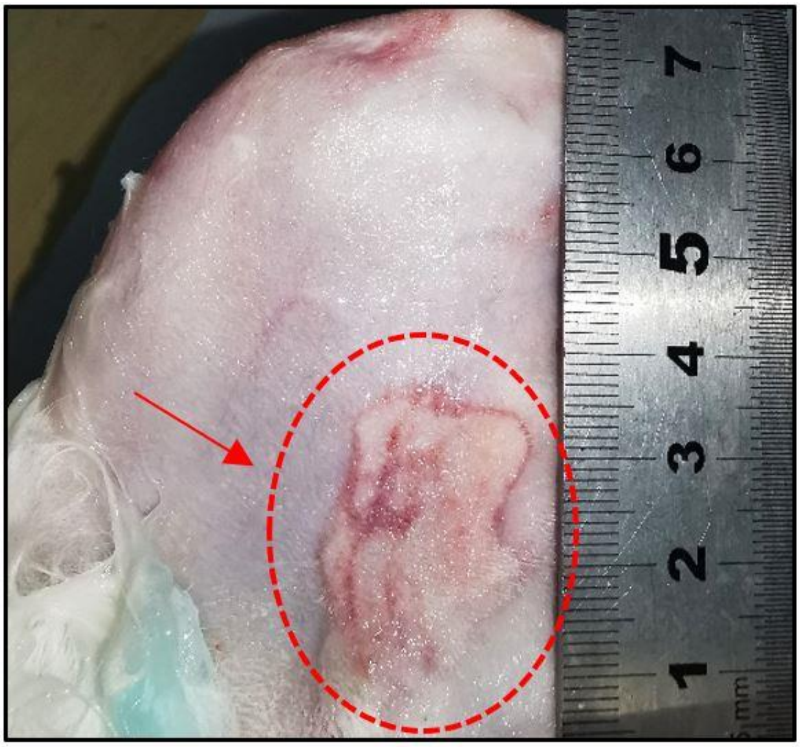

Figure 7: Macroscopic appearance of the overlapping lesions from the inner side of the left thigh and above the skin. The red arrow indicates the area of the created overlapping lesions on the skin.

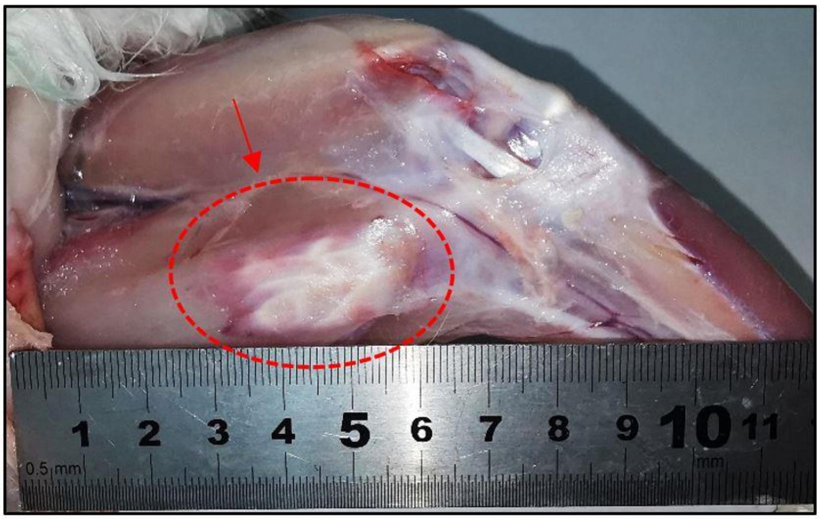

Figure 8: Macroscopic appearance of the exposed muscle from the inner side of the left thigh. Overlapping lesions were formed after performing a 3 x 3 grid at step movement of 5 mm. The red arrow indicates the area of the created overlapping lesions on the exposed muscle.

The muscle of the left thigh was dissected and a necrotic region was observed as shown in Figure 9.

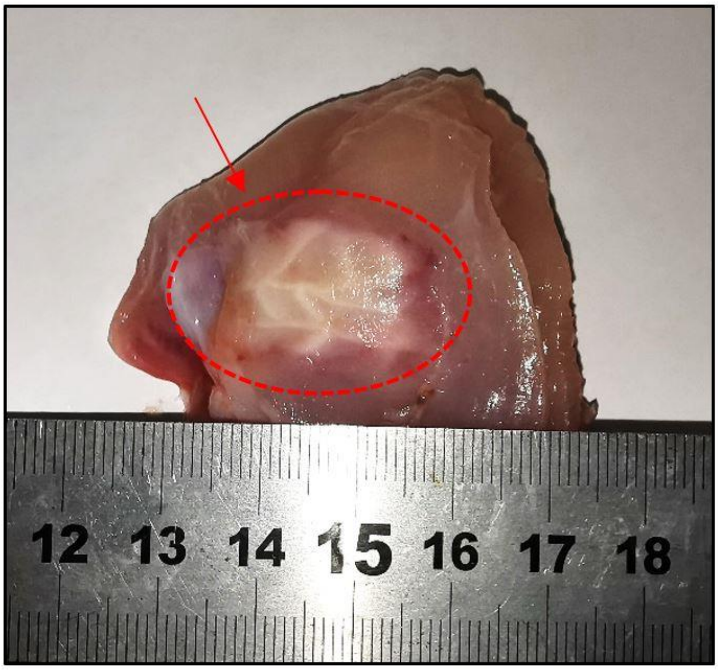

Figure 9: Macroscopic appearance of the overlapping lesions (indicated with red arrow) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 9 overlapping lesions on the surface of the muscle was calculated to be 19 mm x 15 mm while its depth was 10 mm. Figure 10 demonstrates the vertical cross middle dissection of the necrotic area that was created on the left thigh by applying a 3 x 3 grid pattern.

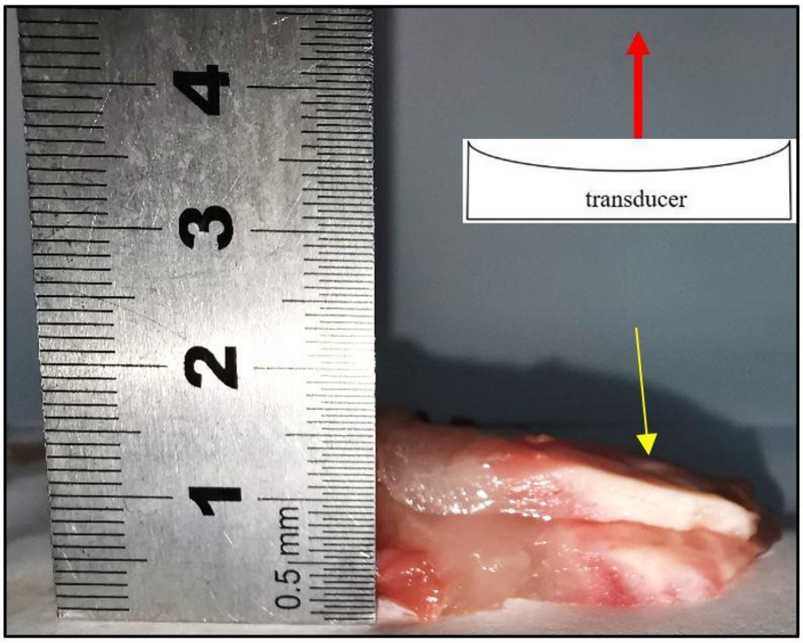

Figure 10: In situ dissection of the overlapping lesions that were created on the left thigh. The yellow arrow indicates the necrotic area and the red arrow indicates the beam direction.

As shown in Figure 11 and Figure 12, no lesions were appeared on the outer side of the right thigh above and below the skin, respectively.

Figure 11: The outer side of the right thigh above the skin after the treatment.

Figure 12: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed.

The lesions were observed to be overlapped on the inner side of the right thigh above the skin and discrete below the skin as shown in Figure 13 and Figure 14, respectively.

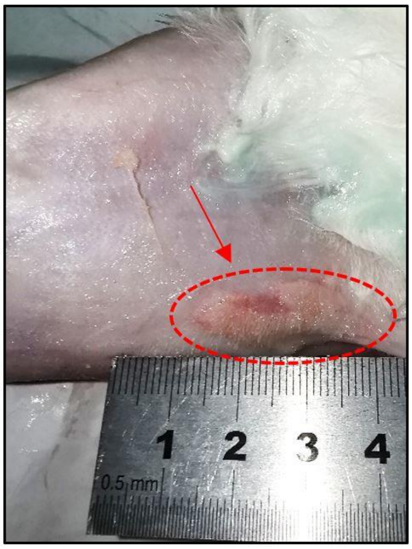

Figure 13: Macroscopic appearance of the overlapping lesions as appeared from the inner side of the right thigh and above the skin. The red arrow indicates the area of the created overlapping lesions on the skin.

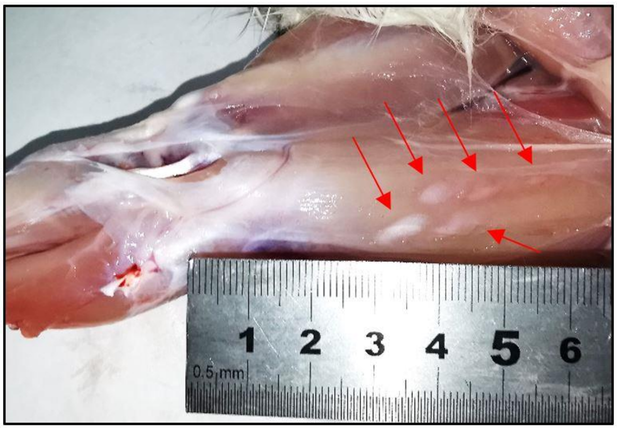

Figure 14: Macroscopic appearance of the discrete lesions on the exposed muscle from the inner side of the right thigh. The red arrows indicate the formed lesions after performing a 3 x 3 grid at step movement of 5 mm.

The muscle of the inner side of the right thigh was dissected and four (4) discrete lesions were appeared as shown in Figure 15.

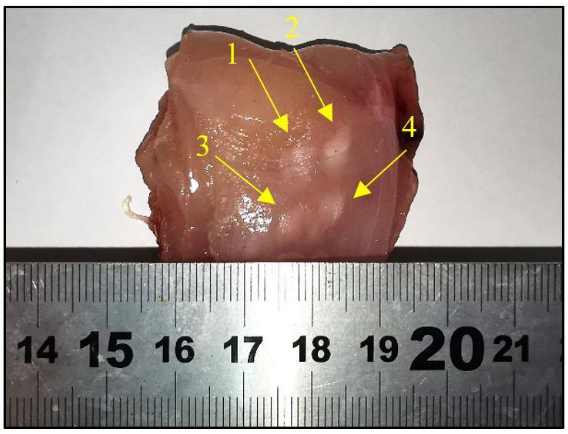

Figure 15: Macroscopic appearance of the formed lesions (indicated with yellow arrows) on the dissected muscle of the inner side of the right thigh (plane perpendicular to the beam).

The depth of the formed lesions was calculated to be 5 mm, 6 mm, 3 mm, and 1 mm for lesion 1, 2, 3, and 4, respectively. Figure 16 demonstrates the dissection of the lesions 1 and 2 that were created on the right thigh and Figure 17 demonstrates the dissection of the lesions 3 and 4.

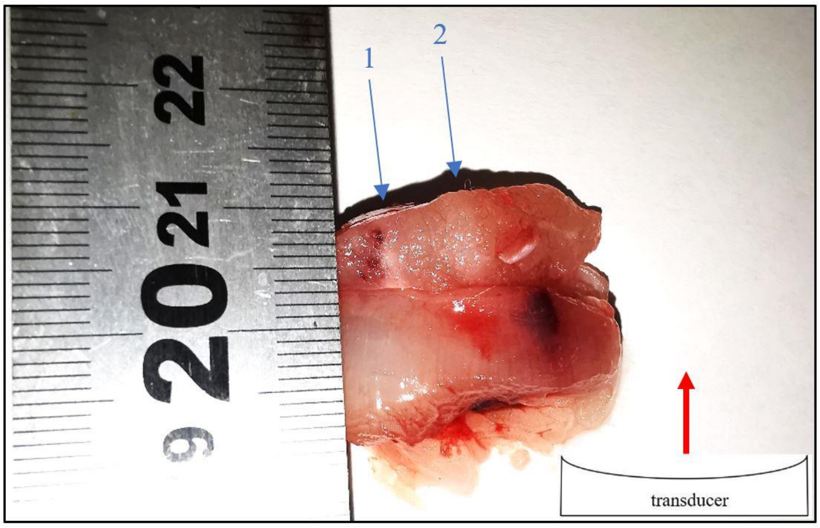

Figure 16: In situ dissection of the formed lesions 1 and 2 of the right thigh. The blue arrows indicate the formed lesions and the red arrow indicates the beam direction.

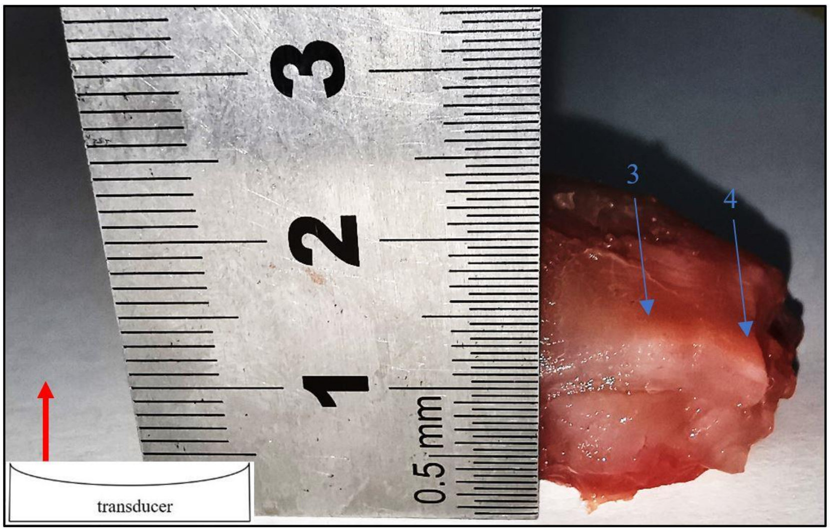

Figure 17: In situ dissection of the formed lesions 3 and 4 of the right thigh. The blue arrows indicate the formed lesions and the red arrow indicates the beam direction.

Four (4) lesions were observed on the dissected muscle of the right thigh after the treatment (Table 3). The diameter and length of the four lesions was measurable.

Table 3: Summary of results for the right thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	5	5
2	4	6
3	4	3
4	3.5	1

Table 4 lists the anesthesia record for the rabbit experiment.

Table 4: Anesthesia record for rabbit 7.

Animal ID	SOUNDPET_2020_7
Species	Rabbit
Sex	M
Age	Adult
Weight (Kg)	2
Supplier	S. Ioannou Farm (CYCU5.05)
Grant	SOUNDPET (INTEGRATED/0918/0008)
Study	CY/EXP/PR.L01/2020
Participants	Kyriakos Spanoudes
	Theocharis Drakos
	Nikolas Evripidou
Principal Investigator	Christakis Damianou
Time	**Anesthesia**
09:30	Medetomidine 1 ml + Ketamine 0.3 ml
10:10	Medetomidine 0.5 ml + Ketamine 0.15 ml
Euthanasia	10:30 1 ml T-61

Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
09:30	100	60	2	Absent	n/a
09:45	110	64	0	Absent	n/a
10:00	100	64	0	Absent	n/a
10:15	100	60	0	Absent	n/a

Rabbit 8

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	250
Acoustic power (W)	77.5
Sonication time (s)	10
Energy (J)	775
Cooling time (s)	90
Number of sonications	9
Step between sonications (mm)	5
Distance transducer/rabbit skin (cm)	7

A 3 x 3 grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 9 at step movement of 5 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

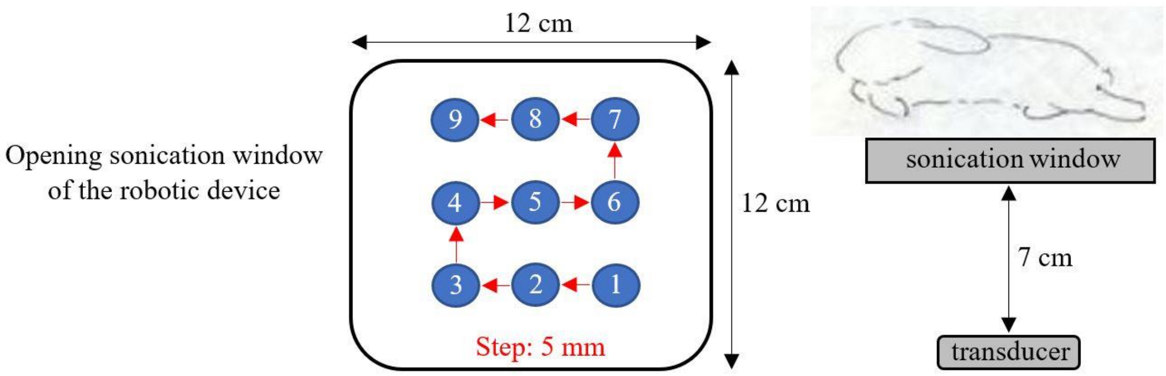

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	250
Acoustic power (W)	77.5
Sonication time (s)	5
Energy (J)	387.5
Cooling time (s)	90
Number of sonications	9
Step between sonications (mm)	5
Distance transducer/rabbit skin (cm)	7

A 3 x 3 grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved from point 1 to 9 at step movement of 5 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

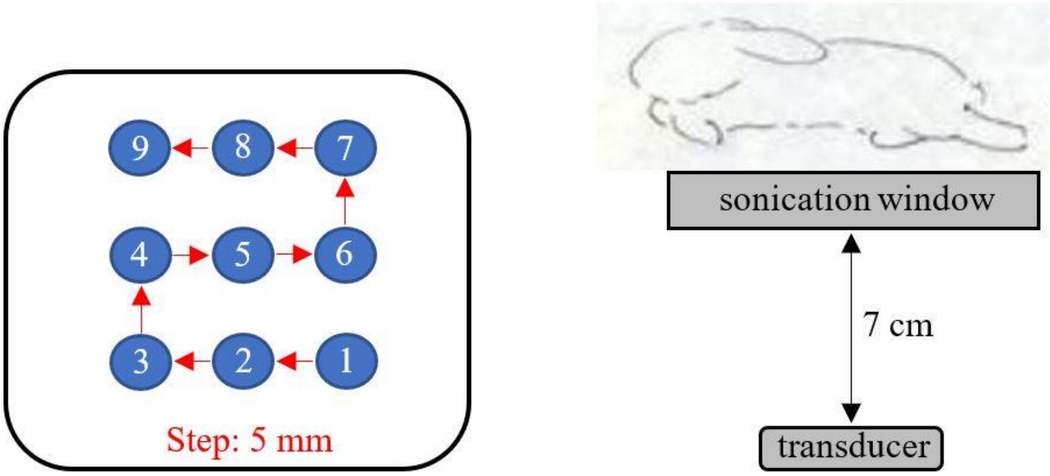

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory that was used for the rabbit thigh ablations.

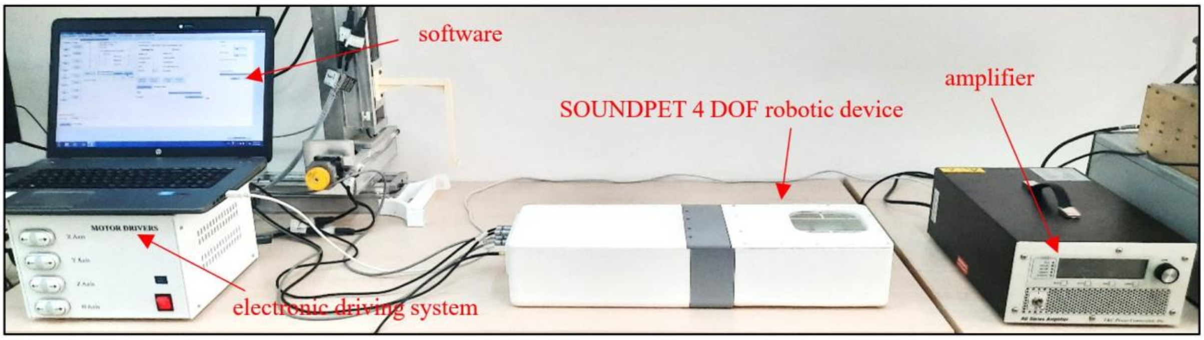

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

Figure 4: Animal positioning for the experiment.

Results

No lesions were appeared from the side of the sonication (outer side) on the left thigh above and below the skin as shown in Figure 5 and Figure 6, respectively.

Figure 5: The outer side of the left thigh above the skin after the treatment.

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side).

Overlapping lesions were appeared on the inner side of the left thigh above and below the skin as shown in Figure 7 and Figure 8, respectively.

Figure 7: Macroscopic appearance of the overlapping lesions from the inner side of the left thigh and above the skin. The red arrow indicates the area of the created overlapping lesions on the skin.

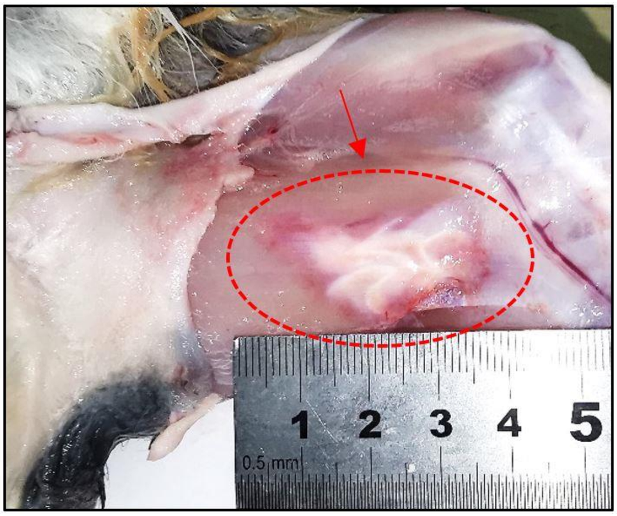

Figure 8: Macroscopic appearance of the exposed muscle from the inner side of the left thigh. Overlapping lesions were formed after performing a 3 x 3 grid at step movement of 5 mm. The red arrow indicates the area of the created overlapping lesions on the exposed muscle.

The muscle of the left thigh was dissected and a necrotic region was observed as shown in Figure 9.

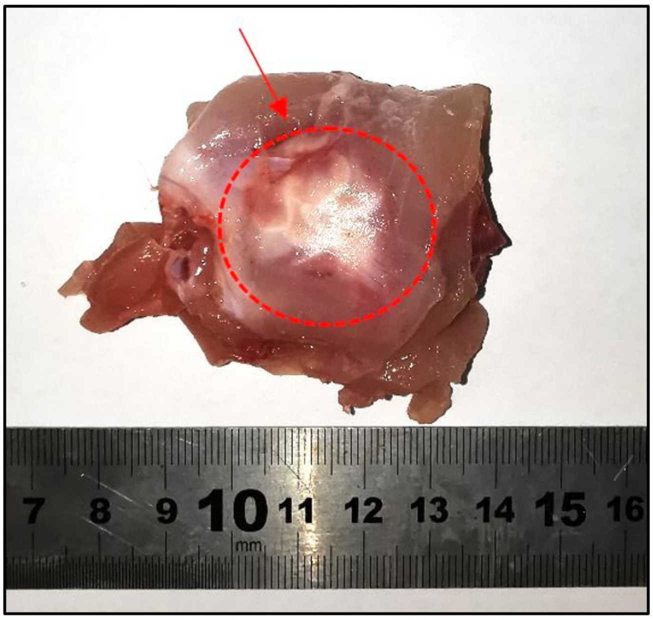

Figure 9: Macroscopic appearance of the overlapping lesions (indicated with red arrow) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 9 overlapping lesions on the surface of the muscle was calculated to be 16 mm x 14 mm while its depth was 8 mm. Figure 10 demonstrates the vertical cross middle dissection of the necrotic area that was created on the left thigh by applying a 3 x 3 grid pattern.

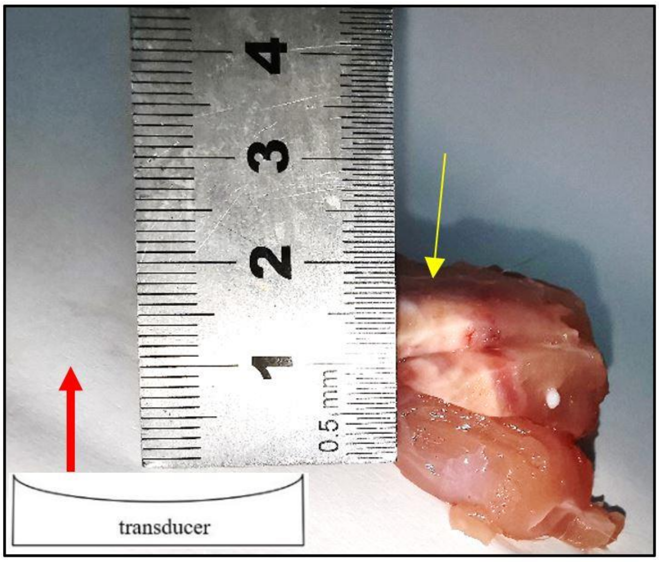

Figure 10: In situ dissection of the overlapping lesions that were created on the left thigh. The yellow arrow indicates the necrotic area and the red arrow indicates the beam direction.

As shown in Figure 11 and Figure 12, no lesions were appeared on the outer side of the right thigh above and below the skin, respectively.

Figure 11: The outer side of the right thigh above the skin after the treatment.

Figure 12: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed.

The lesions were observed to be overlapped on the inner side of the right thigh above the skin and discrete (but very faintly) below the skin as shown in Figure 13 and Figure 14, respectively.

Figure 13: Macroscopic appearance of the overlapping lesions as appeared from the inner side of the right thigh and above the skin. The red arrow indicates the area of the created overlapping lesions on the skin.

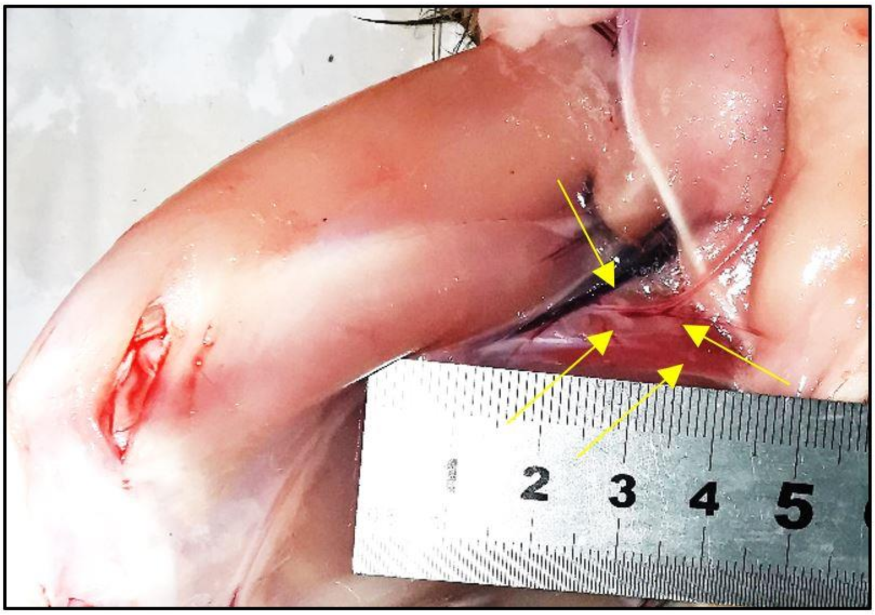

Figure 14: Macroscopic appearance of the discrete lesions on the exposed muscle from the inner side of the right thigh. The yellow arrows indicate the formed lesions after performing a 3 x 3 grid at step movement of 5 mm.

The muscle of the inner side of the right thigh was dissected and the lesions were very faintly as shown in Figure 15. Moreover, they were formed very close to the bone making them impossible to be dissected.

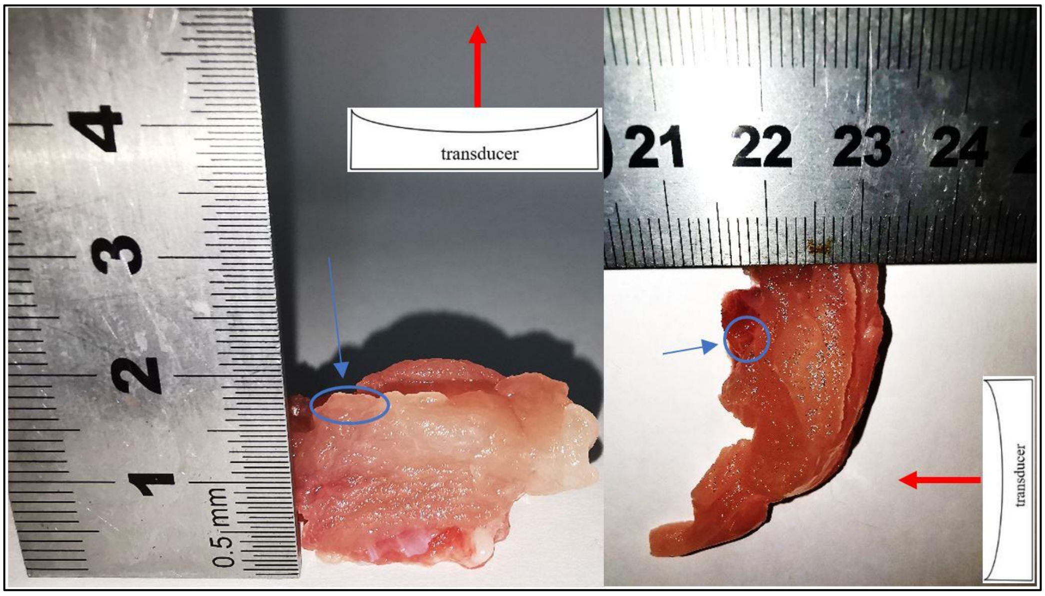

Figure 15: In situ dissection of the discrete lesions that could barely been observed on the right thigh. The blue arrows indicate the lesions and the red arrow indicates the beam direction.

Table 3 lists the anesthesia record for the rabbit experiment.

Table 3: Anesthesia record for rabbit 8.

Animal ID	SOUNDPET_2020_8
Species	Rabbit
Sex	F
Age	Adult
Weight (Kg)	2.1
Supplier	S. Ioannou Farm (CYCU5.05)
Grant	SOUNDPET (INTEGRATED/0918/0008)
Study	CY/EXP/PR.L01/2020
Participants	Kyriakos Spanoudes
	Theocharis Drakos
	Nikolas Evripidou
Principal Investigator	Christakis Damianou
Time	**Anesthesia**
10:30	Medetomidine 1 ml + Ketamine 0.3 ml
11:10	Medetomidine 0.5 ml + Ketamine 0.15 ml
Euthanasia	11:40 1 ml T-61

Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
10:30	98	62	0	Absent	n/a
10:45	102	62	0	Absent	n/a
11:00	102	68	1	Absent	n/a
11:15	110	68	0	Absent	n/a
11:30	106	64	0	Absent	n/a

Rabbit 9

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	300
Acoustic power (W)	93
Sonication time (s)	10
Energy (J)	930
Cooling time (s)	90
Number of sonications	9
Step between sonications (mm)	5
Distance transducer/rabbit skin (cm)	7

A 3 x 3 grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 9 at step movement of 5 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

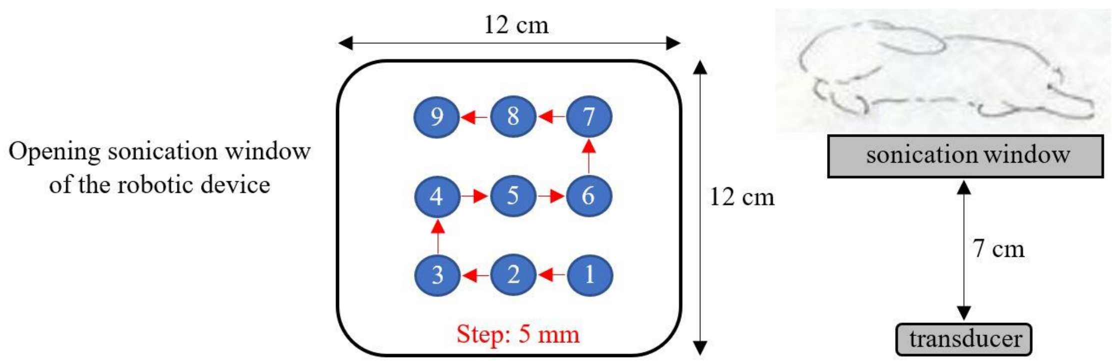

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	300
Acoustic power (W)	93
Sonication time (s)	5
Energy (J)	465
Cooling time (s)	90
Number of sonications	9
Step between sonications (mm)	5
Distance transducer/rabbit skin (cm)	7

A 3 x 3 grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved from point 1 to 9 at step movement of 5 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

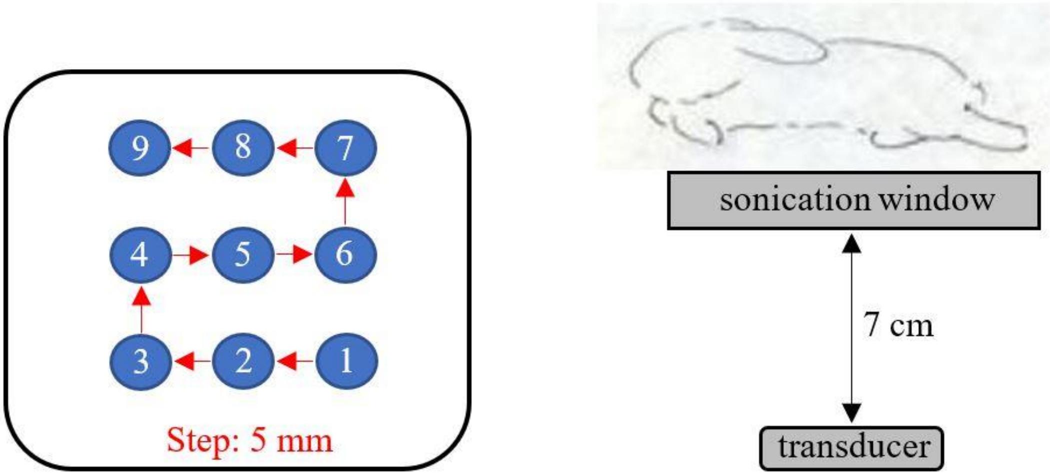

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory that was used for the rabbit thigh ablations.

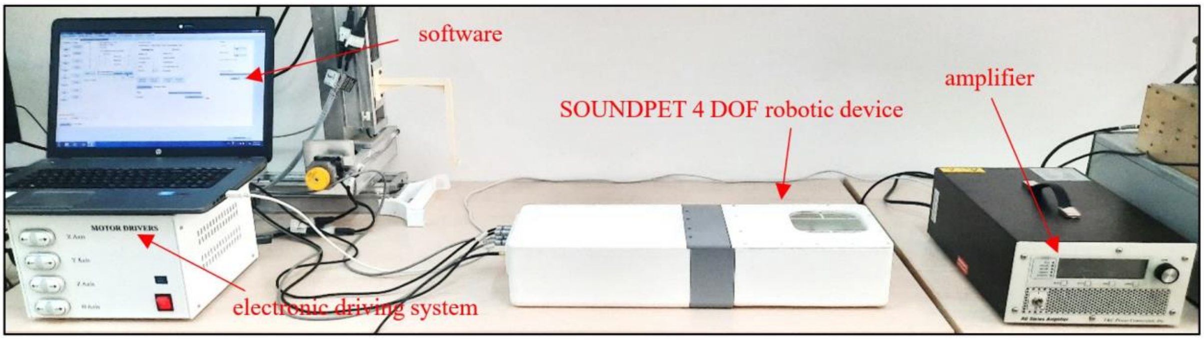

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

Figure 4: Animal positioning for the experiment.

Results

A necrotic area (faint red marks) was observed from the side of the sonication (outer side) on the left thigh above the skin as shown in Figure 5. No lesions were observed below the skin as shown in Figure 6.

Figure 5: The outer side of the left thigh above the skin after the treatment. The red arrow indicates the area where the necrotic region was appeared.

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side).

Overlapping lesions were appeared on the inner side of the left thigh above and below the skin as shown in Figure 7 and Figure 8, respectively.

Figure 7: Macroscopic appearance of the overlapping lesions from the inner side of the left thigh and above the skin. The red arrow indicates the area of the created overlapping lesions on the skin.

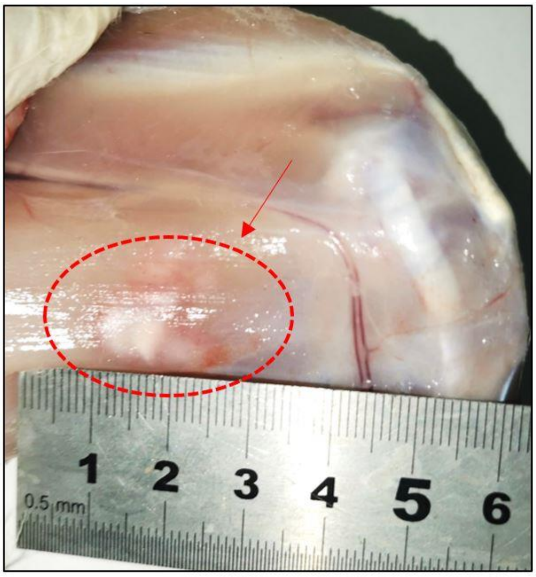

Figure 8: Macroscopic appearance of the exposed muscle from the inner side of the left thigh. Overlapping lesions were formed after performing a 3 x 3 grid at step movement of 5 mm. The red arrow indicates the area of the created overlapping lesions on the exposed muscle.

The muscle of the left thigh was dissected and a necrotic region was observed as shown in Figure 9.

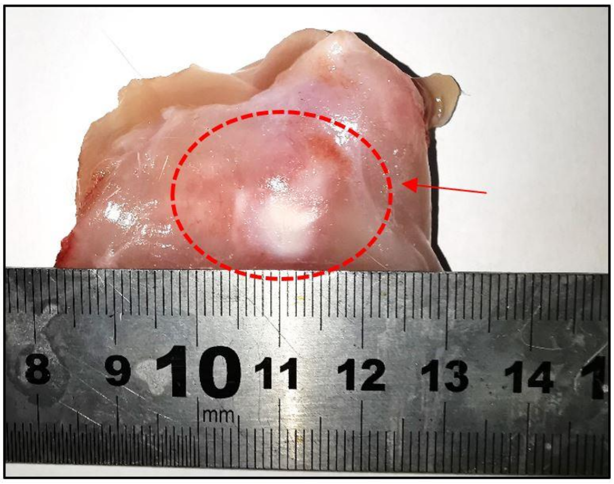

Figure 9: Macroscopic appearance of the overlapping lesions (indicated with red arrow) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 9 overlapping lesions on the surface of the muscle was calculated to be 15 mm x 15 mm while its depth was 7 mm. Figure 10 demonstrates the vertical cross middle dissection of the necrotic area that was created on the left thigh by applying a 3 x 3 grid pattern.

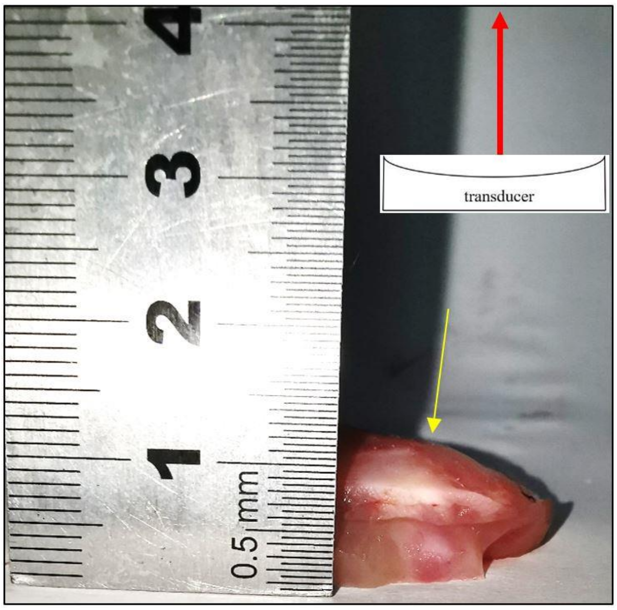

Figure 10: In situ dissection of the overlapping lesions that were created on the left thigh. The yellow arrow indicates the necrotic area and the red arrow indicates the beam direction.

As shown in Figure 11 and Figure 12, no lesions were appeared on the outer side of the right thigh above and below the skin, respectively.

Figure 11: The outer side of the right thigh above the skin after the treatment.

Figure 12: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed.

The lesions were observed to be overlapped on the inner side of the right thigh above and below the skin as shown in Figure 13 and Figure 14, respectively.

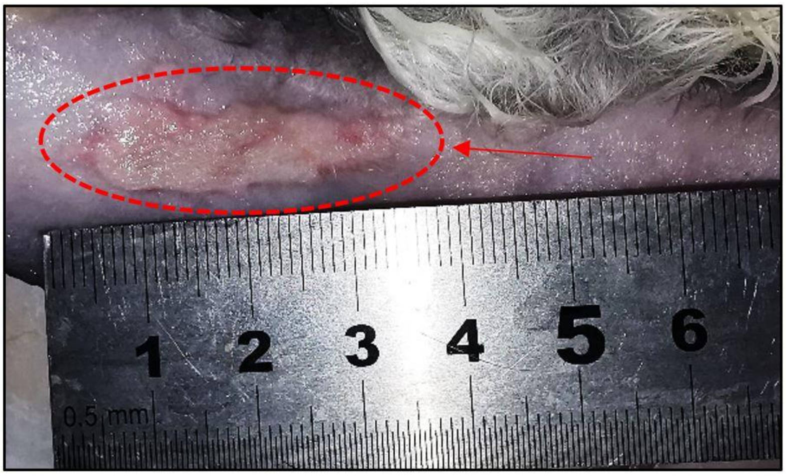

Figure 13: Macroscopic appearance of the overlapping lesions as observed from the inner side of the right thigh and above the skin. The red arrow indicates the area of the created overlapping lesions on the skin.

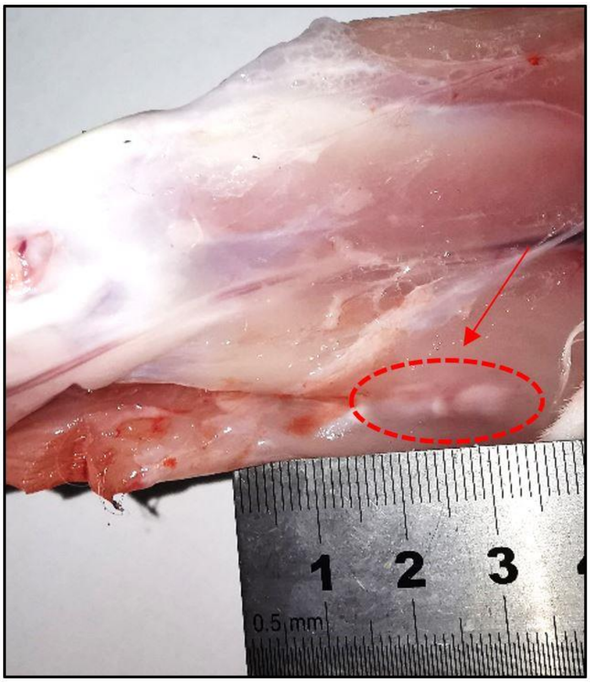

Figure 14: Macroscopic appearance of the overlapping lesions on the exposed muscle from the inner side of the right thigh. The red arrow indicates the formed lesions after performing a 3 x 3 grid at step movement of 5 mm.

The muscle of the inner side of the right thigh was dissected and a necrotic region was observed as shown in Figure 15.

Figure 15: Macroscopic appearance of the formed lesions (indicated with red arrow) on the dissected muscle of the inner side of the right thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 9 overlapping lesions on the surface of the muscle was calculated to be 19 mm x 9 mm while its depth was 2 mm. Figure 16 demonstrates the vertical cross middle dissection of the necrotic area that was created on the left thigh by applying a 3 x 3 grid pattern.

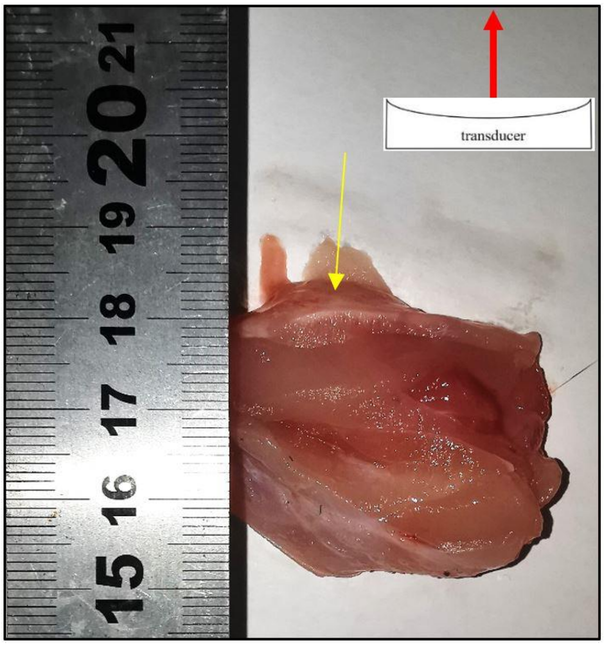

Figure 16: In situ dissection of the overlapping lesions that were created on the right thigh. The yellow arrow indicates the necrotic area and the red arrow indicates the beam direction.

Table 3 lists the anesthesia record for the rabbit experiment.

Table 3: Anesthesia record for rabbit 9.

Animal ID	SOUNDPET_2020_9
Species	Rabbit
Sex	M
Age	Adult
Weight (Kg)	1.9
Supplier	S. Ioannou Farm (CYCU5.05)
Grant	SOUNDPET (INTEGRATED/0918/0008)
Study	CY/EXP/PR.L01/2020
Participants	Kyriakos Spanoudes
	Theocharis Drakos
	Nikolas Evripidou
Principal Investigator	Christakis Damianou
Time	**Anesthesia**
09:25	Medetomidine 1 ml + Ketamine 0.3 ml
10:00	Medetomidine 0.5 ml + Ketamine 0.15 ml
Euthanasia	10:25 1 ml T-61

Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
09:30	98	60	0	Absent	n/a
09:40	102	60	0	Absent	n/a

09:50	98	48	1	Absent	n/a
10:00	96	60	0	Absent	n/a
10:10	92	56	0	Absent	n/a
10:20	96	56	0	Absent	n/a

Rabbit 10

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	300
Acoustic power (W)	93
Sonication time (s)	10
Energy (J)	930
Cooling time (s)	90
Number of sonications	9
Step between sonications (mm)	5
Distance transducer/rabbit skin (cm)	7

A 3 x 3 grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 9 at step movement of 5 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

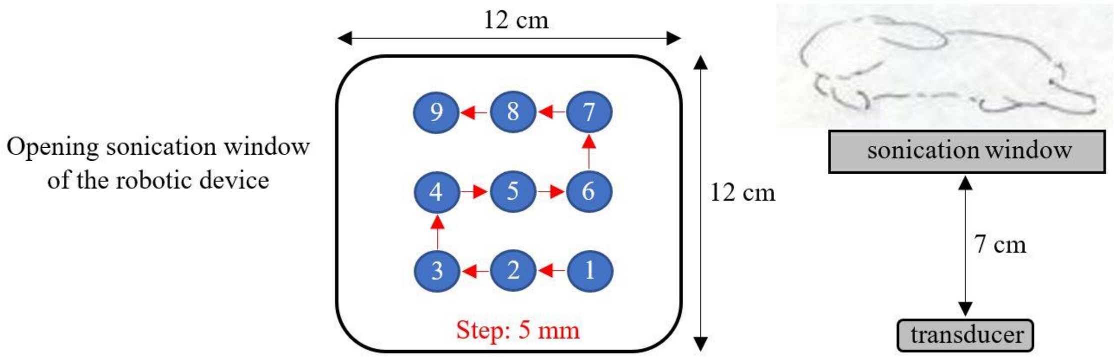

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	250
Acoustic power (W)	77.5
Sonication time (s)	10
Energy (J)	775
Cooling time (s)	90
Number of sonications	9
Step between sonications (mm)	5
Distance transducer/rabbit skin (cm)	7

A 3 x 3 grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved from point 1 to 9 at step movement of 5 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

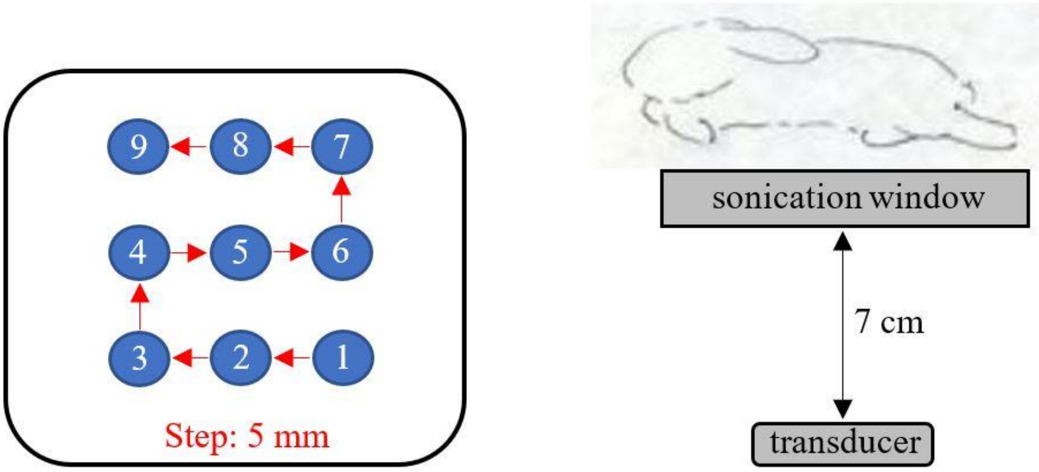

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory that was used for the rabbit thigh ablations.

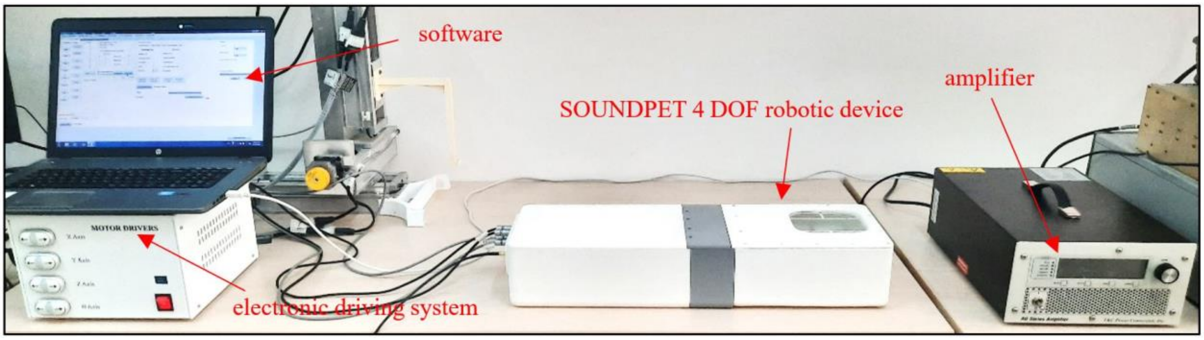

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

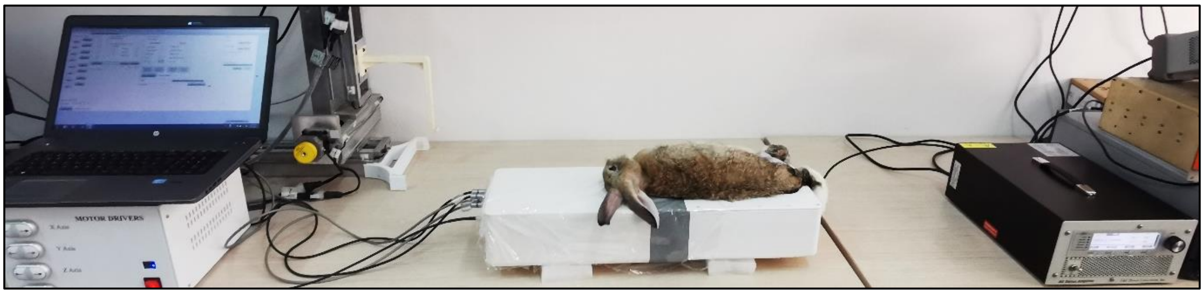

Figure 4: Animal positioning for the experiment.

Results

A necrotic area was observed from the side of the sonication (outer side) on the left thigh above the skin as shown in Figure 5. No lesions were observed below the skin as shown in Figure 6.

Figure 5: The outer side of the left thigh above the skin after the treatment.

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side).

Overlapping lesions were appeared on the inner side of the left thigh above and below the skin as shown in Figure 7 and Figure 8, respectively.

Figure 7: Macroscopic appearance of the overlapping lesions from the inner side of the left thigh and above the skin. The red arrow indicates the area of the created overlapping lesions on the skin.

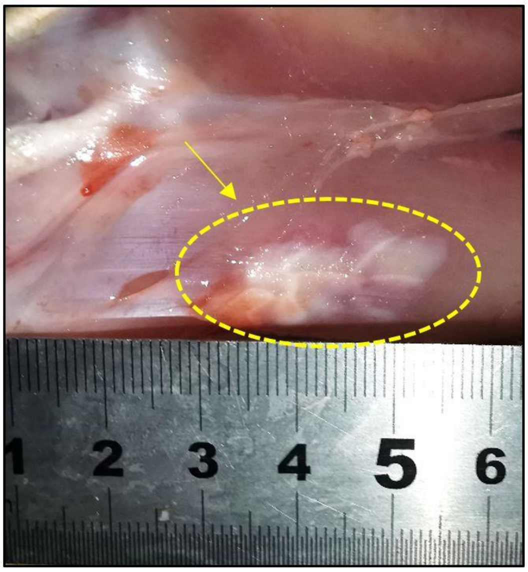

Figure 8: Macroscopic appearance of the exposed muscle from the inner side of the left thigh. Overlapping lesions were formed after performing a 3 x 3 grid at step movement of 5 mm. The yellow arrow indicates the area of the created overlapping lesions on the exposed muscle.

The muscle of the left thigh was dissected and a necrotic region was observed as shown in Figure 9.

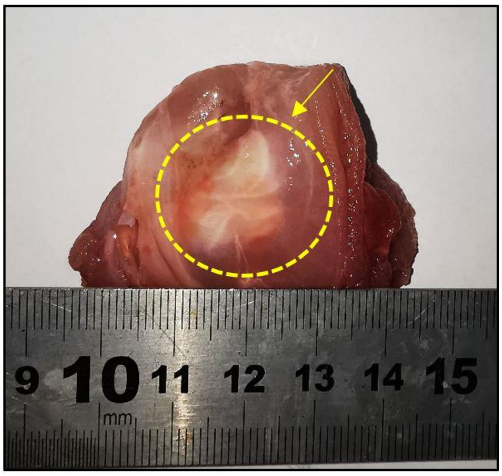

Figure 9: Macroscopic appearance of the overlapping lesions (indicated with yellow arrow) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 9 overlapping lesions on the surface of the muscle was calculated to be 15 mm x 17 mm while its depth was 14 mm. Figure 10 demonstrates the vertical cross middle dissection of the necrotic area that was created on the left thigh by applying a 3 x 3 grid pattern.

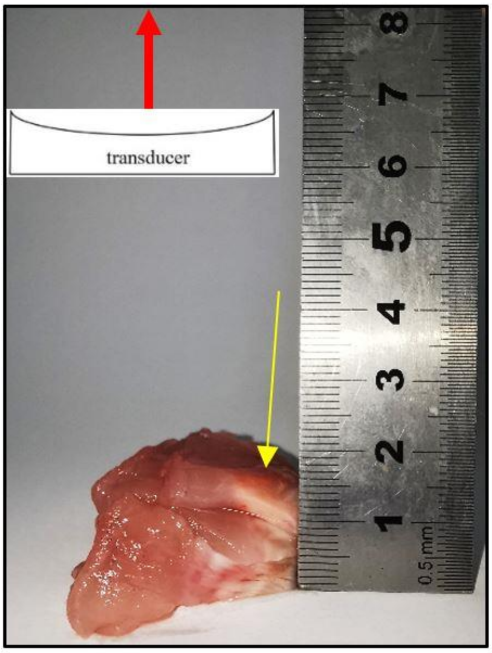

Figure 10: In situ dissection of the overlapping lesions that were created on the left thigh. The yellow arrow indicates the necrotic area and the red arrow indicates the beam direction.

A necrotic area was observed from the side of the sonication (outer side) on the right thigh above the skin as shown in Figure 11. No lesions were observed below the skin as shown in Figure 12.

Figure 11: The outer side of the right thigh above the skin after the treatment.

Figure 12: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed.

Overlapping lesions were appeared on the inner side of the right thigh above and below the skin as shown in Figure 13 and Figure 14, respectively.

Figure 13: Macroscopic appearance of the overlapping lesions as observed from the inner side of the right thigh and above the skin. The red arrow indicates the area of the created overlapping lesions on the skin.

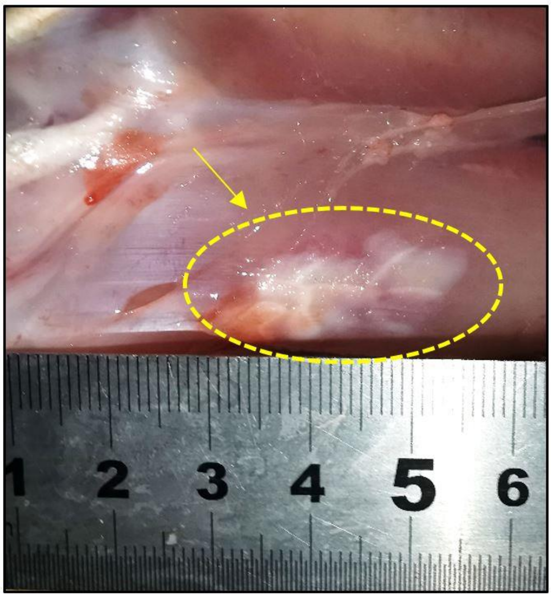

Figure 14: Macroscopic appearance of the overlapping lesions on the exposed muscle from the inner side of the right thigh. The yellow arrow indicates the formed lesions after performing a 3 x 3 grid at step movement of 5 mm.

The muscle of the right thigh was dissected and a necrotic region was observed as shown in Figure 15.

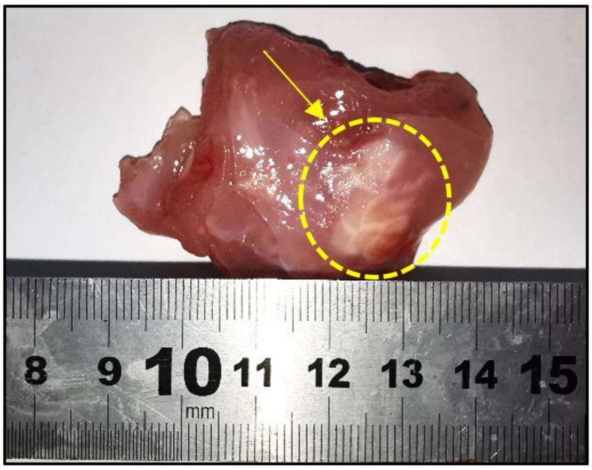

Figure 15: Macroscopic appearance of the formed lesions (indicated with yellow arrows) on the dissected muscle of the inner side of the right thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 9 overlapping lesions on the surface of the muscle was calculated to be 13 mm x 12 mm while its depth was 8 mm. Figure 16 demonstrates the vertical cross middle dissection of the necrotic area that was created on the right thigh by applying a 3 x 3 grid pattern.

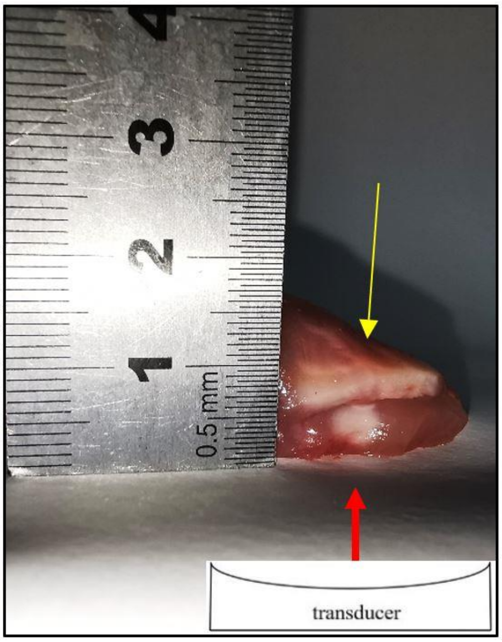

Figure 16: In situ dissection of the overlapping lesions on the right thigh. The yellow arrow indicates the formed lesions and the red arrow indicates the beam direction.

Table 3 lists the anesthesia record for the rabbit experiment.

Table 3: Anesthesia record for rabbit 10.

Animal ID	SOUNDPET_2020_10				
Species	Rabbit				
Sex	M				
Age	Adult				
Weight (Kg)	2.1				
Supplier	S. Ioannou Farm (CYCU5.05)				
Grant	SOUNDPET (INTEGRATED/0918/0008)				
Study	CY/EXP/PR.L01/2020				
Participants	Kyriakos Spanoudes				
	Theocharis Drakos				
	Nikolas Evripidou				
Principal Investigator	Christakis Damianou				
Time	**Anesthesia**				
12:00	Medetomidine 1 ml + Ketamine 0.3 ml				
12:35	Medetomidine 0.5 ml + Ketamine 0.15 ml				
Euthanasia	12:50 1 ml T-61				
Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
12:00	110	60	0	Absent	n/a
12:10	100	72	0	Absent	n/a
12:20	110	72	0	Absent	n/a

12:30	110	68	0	Absent	n/a
12:40	114	68	0	Absent	n/a
12:50	118	72	1	Absent	n/a

Rabbit 11

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	300
Acoustic power (W)	93
Sonication time (s)	5
Energy (J)	465
Cooling time (s)	90
Number of sonications	16
Step between sonications (mm)	5
Distance transducer/rabbit skin (cm)	7

A 4 x 4 grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 16 at step movement of 5 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

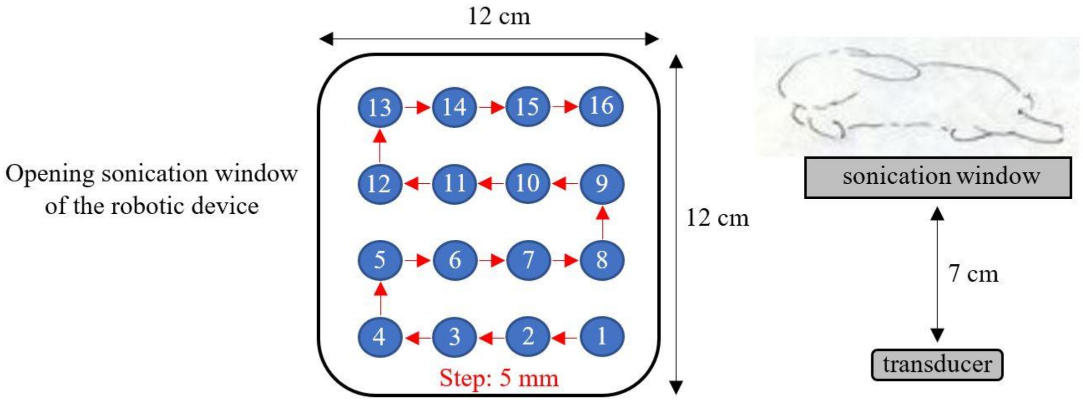

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	300
Acoustic power (W)	93
Sonication time (s)	3
Energy (J)	279
Cooling time (s)	90
Number of sonications	16
Step between sonications (mm)	5
Distance transducer/rabbit skin (cm)	7

A 4 x 4 grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved from point 1 to 16 at step movement of 5 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

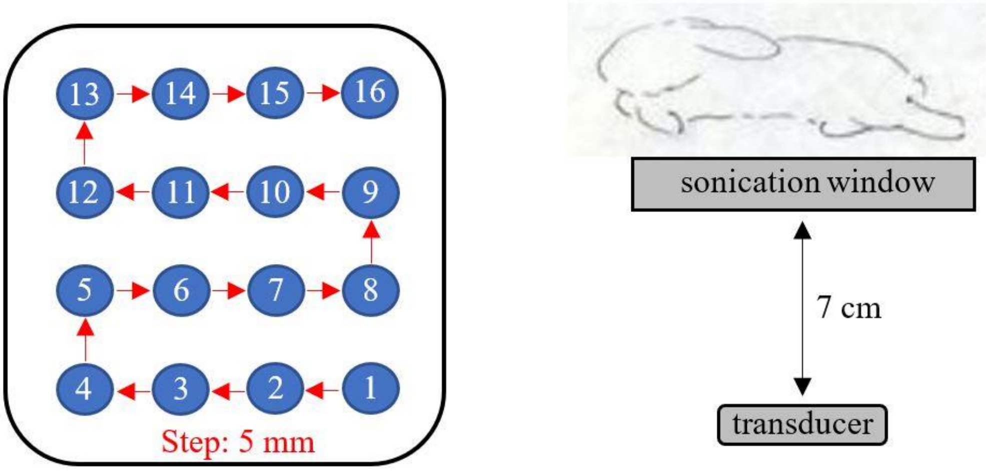

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory that was used for the rabbit thigh ablations.

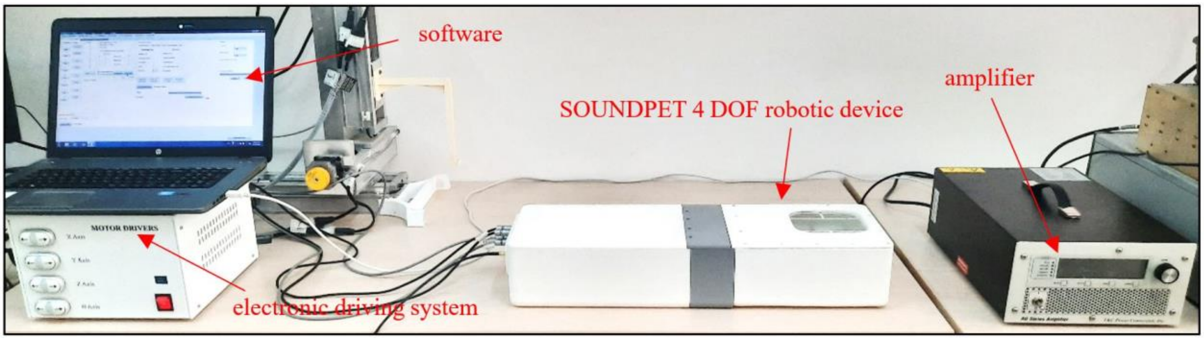

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

Figure 4: Animal positioning for the experiment.

Results

A necrotic area was observed from the side of the sonication (outer side) on the left thigh above the skin as shown in Figure 5. No lesions were observed below the skin as shown in Figure 6.

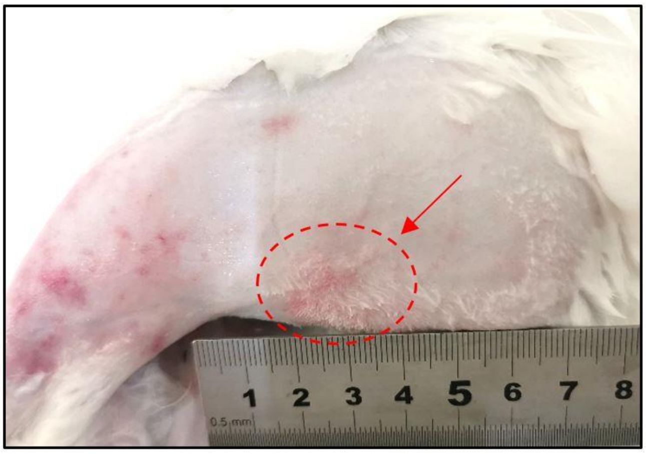

Figure 5: The outer side of the left thigh above the skin after the treatment.

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side).

Overlapping lesions were appeared on the inner side of the left thigh above and below the skin as shown in Figure 7 and Figure 8, respectively.

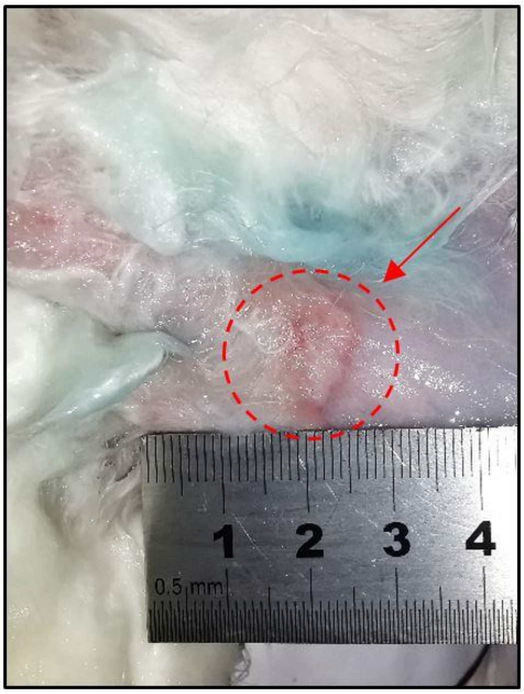

Figure 7: Macroscopic appearance of the overlapping lesions from the inner side of the left thigh and above the skin. The red arrow indicates the area of the created overlapping lesions on the skin.

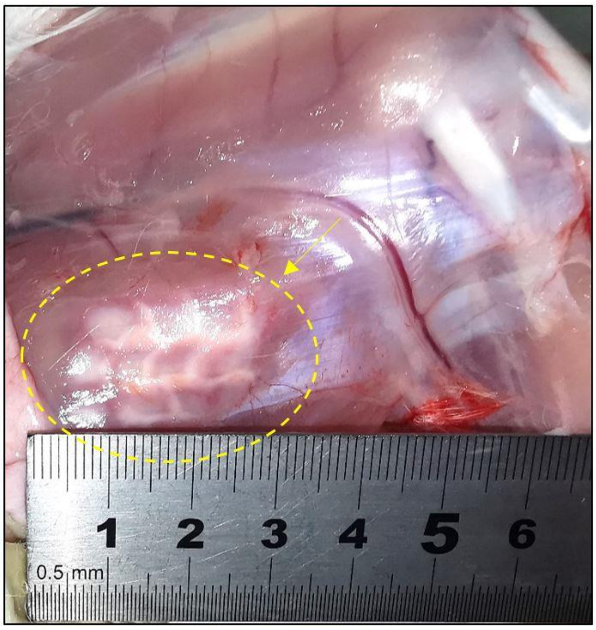

Figure 8: Macroscopic appearance of the exposed muscle from the inner side of the left thigh. Overlapping lesions were formed after performing a 4 x 4 grid at step movement of 5 mm. The yellow arrow indicates the area of the created overlapping lesions on the exposed muscle.

The muscle of the left thigh was dissected and a necrotic region was observed as shown in Figure 9.

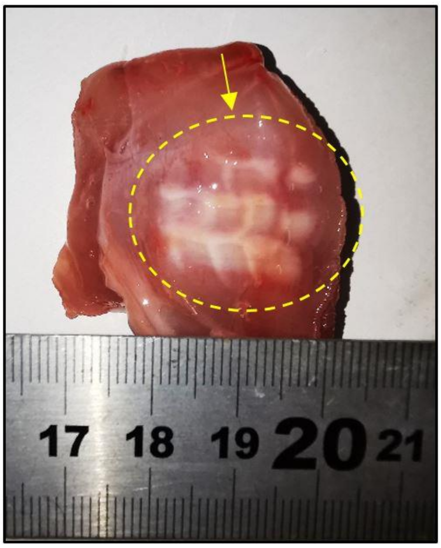

Figure 9: Macroscopic appearance of the overlapping lesions (indicated with yellow arrow) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 16 overlapping lesions on the surface of the muscle was calculated to be 19 mm x 15 mm while its depth was 8 mm. Figure 10 demonstrates the vertical cross middle dissection of the necrotic area that was created on the left thigh by applying a 4 x 4 grid pattern.

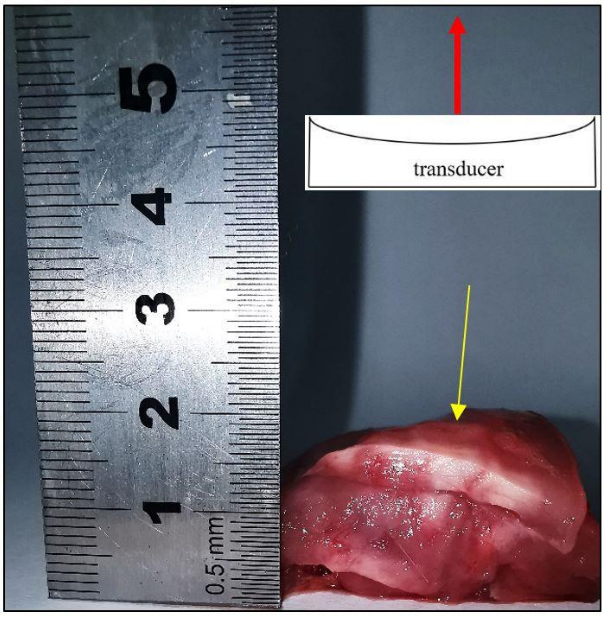

Figure 10: In situ dissection of the overlapping lesions that were created on the left thigh. The yellow arrow indicates the necrotic area and the red arrow indicates the beam direction.

No lesions were observed from the side of the sonication (outer side) on the right thigh above and below the skin as shown in Figure 11 and Figure 12, respectively.

Figure 11: The outer side of the right thigh above the skin after the treatment.

Figure 12: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed.

Overlapping lesions were appeared on the inner side of the right thigh above the skin as shown in Figure 13. Some of the lesions below the skin appeared to be overlapped while some others appeared to be discrete with an average diameter of 5 mm as shown in Figure 14.

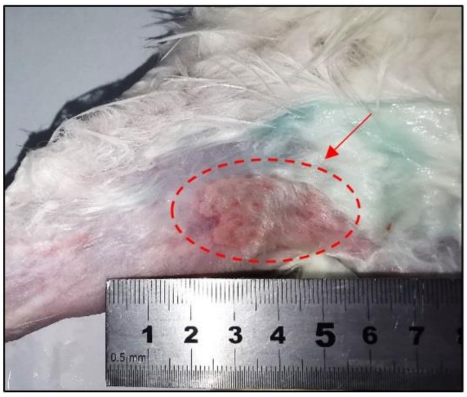

Figure 13: Macroscopic appearance of the overlapping lesions as observed from the inner side of the right thigh and above the skin. The red arrow indicates the area of the created overlapping lesions on the skin.

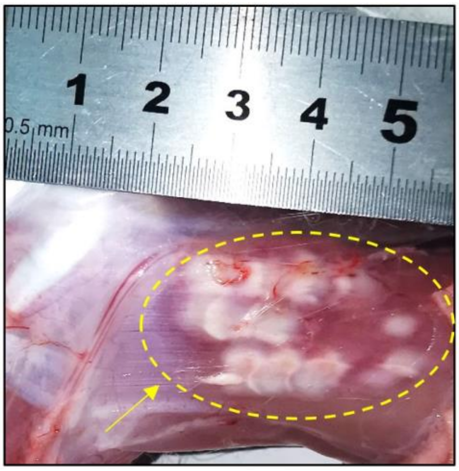

Figure 14: Macroscopic appearance of the overlapping lesions on the exposed muscle from the inner side of the right thigh. The yellow arrow indicates the formed lesions after performing a 4 x 4 grid at step movement of 5 mm.

The muscle of the right thigh was dissected and a necrotic region was observed as shown in Figure 15.

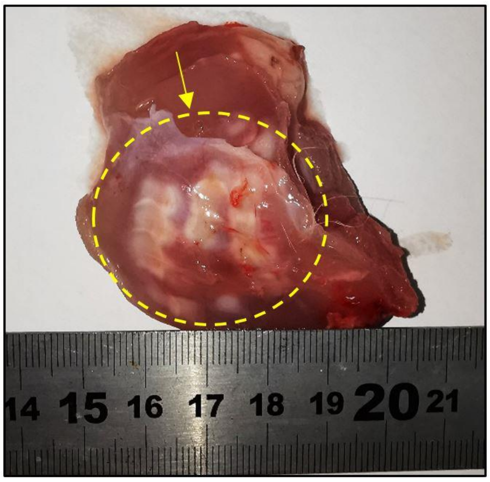

Figure 15: Macroscopic appearance of the formed lesions (indicated with yellow arrows) on the dissected muscle of the inner side of the right thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 16 overlapping lesions on the surface of the muscle was calculated to be 22 mm x 19 mm while its depth was 11 mm. Figure 16 demonstrates the vertical cross middle dissection of the necrotic area that was created on the right thigh by applying a 4 x 4 grid pattern.

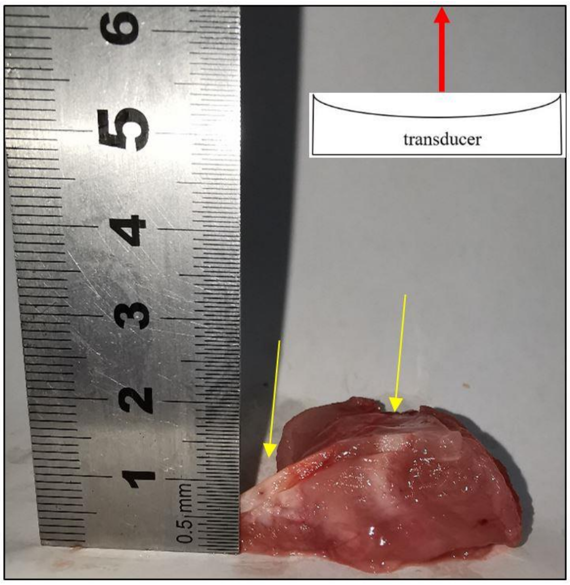

Figure 16: In situ dissection of the overlapping lesions on the right thigh. The yellow arrows indicate the formed lesions and the red arrow indicates the beam direction.

Table 3 lists the anesthesia record for the rabbit experiment.

Table 3: Anesthesia record for rabbit 11.

Animal ID	SOUNDPET_2020_11
Species	Rabbit
Sex	M
Age	Adult
Weight (Kg)	2.2
Supplier	S. Ioannou Farm (CYCU5.05)
Grant	SOUNDPET (INTEGRATED/0918/0008)
Study	CY/EXP/PR.L01/2020
Participants	Kyriakos Spanoudes
	Theocharis Drakos
	Nikolas Evripidou
Principal Investigator	Christakis Damianou
Time	**Anesthesia**
09:35	Medetomidine 1 ml + Ketamine 0.3 ml
10:20	Medetomidine 0.12 ml + Ketamine 0.11 ml
Euthanasia	10:50 1 ml T-61

Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement**	**Temp. (°C)**

				/Pedal Reflex	
09:40	110	60	0	Absent	n/a
09:50	110	52	0	Absent	n/a
10:00	100	56	0	Absent	n/a
10:10	108	56	0	Absent	n/a
10:20	108	60	0	Absent	n/a
10:30	100	60	1	Absent	n/a
10:40	100	56	0	Absent	n/a
10:50	108	56	0	Absent	n/a

Rabbit 12

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	300
Acoustic power (W)	93
Sonication time (s)	5
Energy (J)	465
Cooling time (s)	90
Number of sonications	16
Step between sonications (mm)	4
Distance transducer/rabbit skin (cm)	7

A 4 x 4 grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 16 at step movement of 4 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

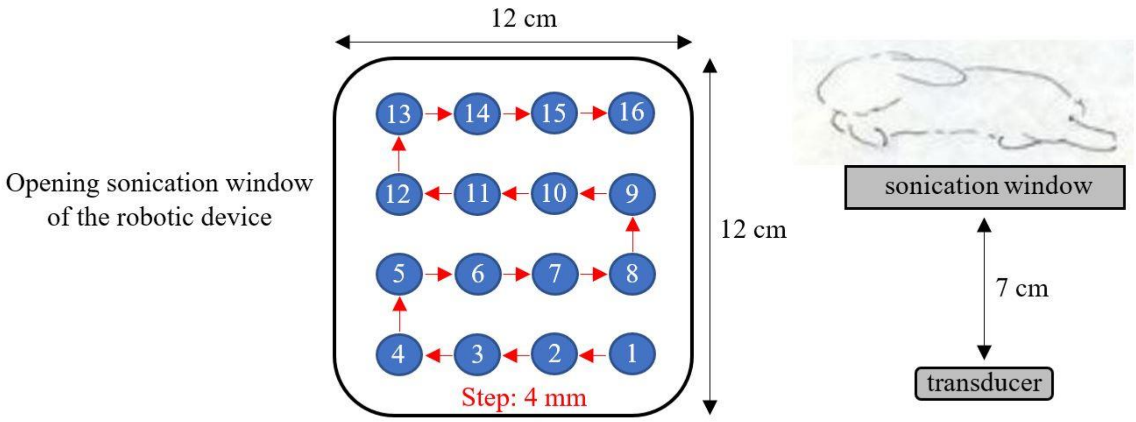

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	300
Acoustic power (W)	93
Sonication time (s)	3
Energy (J)	279
Cooling time (s)	90
Number of sonications	16
Step between sonications (mm)	4
Distance transducer/rabbit skin (cm)	7

A 4 x 4 grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved from point 1 to 16 at step movement of 4 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

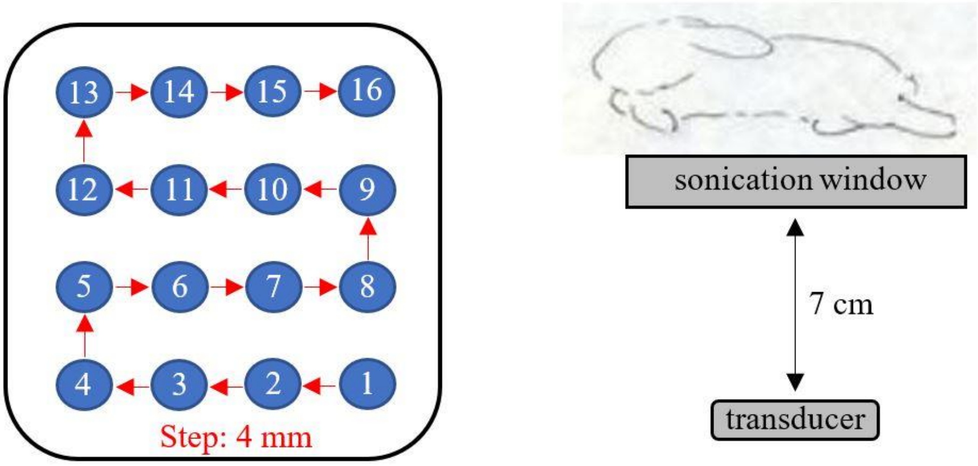

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory that was used for the rabbit thigh ablations.

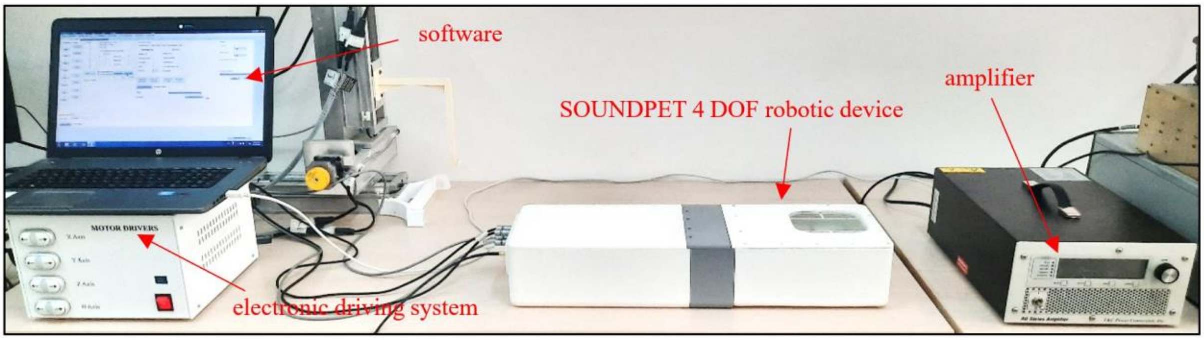

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

Figure 4: Animal positioning for the experiment.

Results

No lesions were observed from the side of the sonication (outer side) on the left thigh above and below the skin as shown in Figure 5 and Figure 6, respectively.

Figure 5: The outer side of the left thigh above the skin after the treatment.

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side).

Overlapping lesions were appeared on the inner side of the left thigh above and below the skin as shown in Figure 7 and Figure 8, respectively.

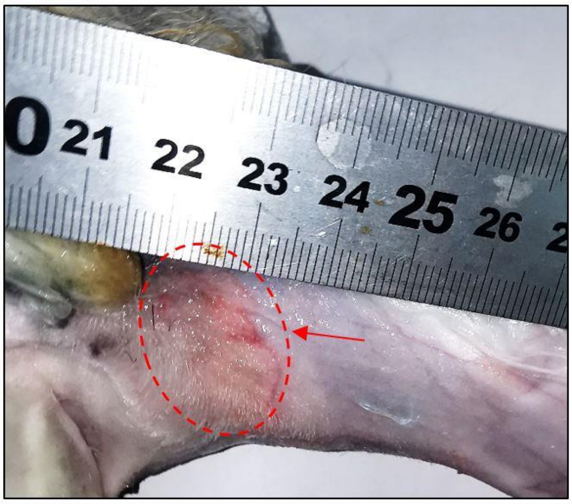

Figure 7: Macroscopic appearance of the overlapping lesions from the inner side of the left thigh and above the skin. The red arrow indicates the area of the created overlapping lesions on the skin.

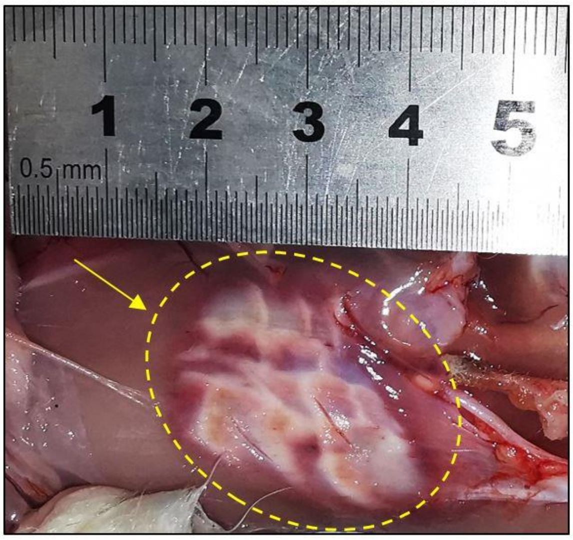

Figure 8: Macroscopic appearance of the exposed muscle from the inner side of the left thigh. Overlapping lesions were formed after performing a 4 x 4 grid at step movement of 4 mm. The yellow arrow indicates the area of the created overlapping lesions on the exposed muscle.

The muscle of the left thigh was dissected and a necrotic region was observed as shown in Figure 9.

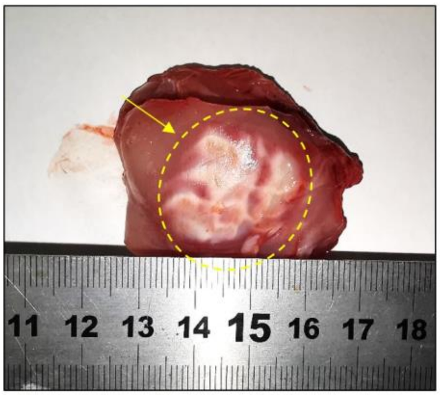

Figure 9: Macroscopic appearance of the overlapping lesions (indicated with yellow arrow) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 16 overlapping lesions on the surface of the muscle was calculated to be 25 mm x 21 mm while its depth was 13 mm. Figure 10 demonstrates the vertical cross middle dissection of the necrotic area that was created on the left thigh by applying a 4 x 4 grid pattern.

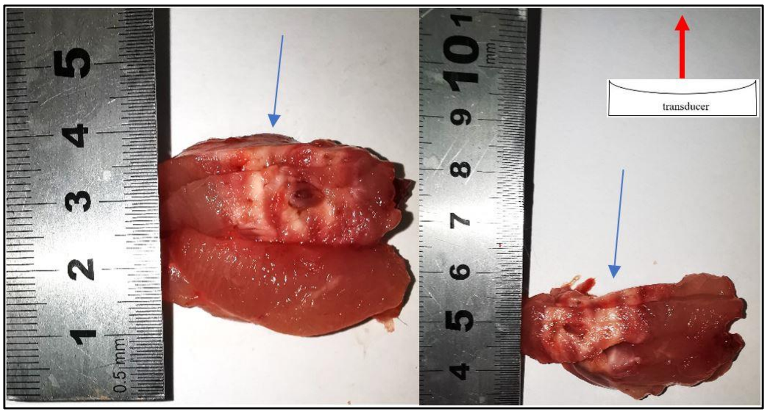

Figure 10: In situ dissection of the overlapping lesions that were created on the left thigh. The blue arrows indicate the necrotic area and the red arrow indicates the beam direction.

No lesions were observed from the side of the sonication (outer side) on the right thigh above and below the skin as shown in Figure 11 and Figure 12, respectively.

Figure 11: The outer side of the right thigh above the skin after the treatment.

Figure 12: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed.

Overlapping lesions were appeared on the inner side of the right thigh above and below the skin as shown in Figure 13 and Figure 14, respectively.

Figure 13: Macroscopic appearance of the overlapping lesions as observed from the inner side of the right thigh and above the skin. The red arrow indicates the area of the created overlapping lesions on the skin.

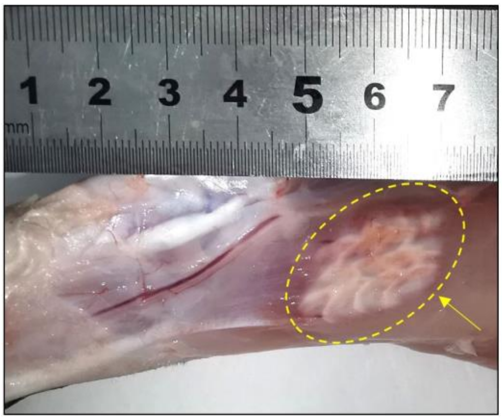

Figure 14: Macroscopic appearance of the overlapping lesions on the exposed muscle from the inner side of the right thigh. The yellow arrow indicates the formed lesions after performing a 4 x 4 grid at step movement of 4 mm.

The muscle of the right thigh was dissected and a necrotic region was observed as shown in Figure 15.

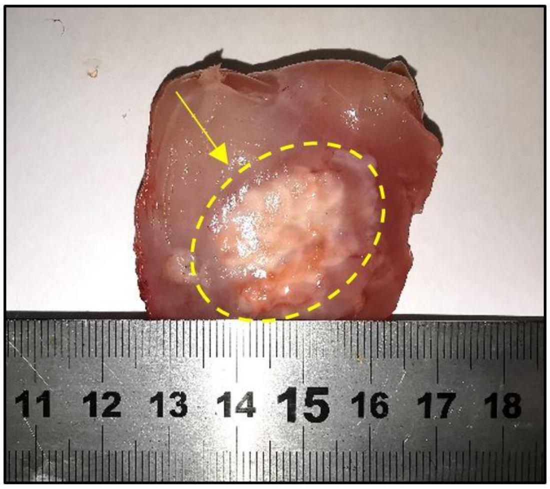

Figure 15: Macroscopic appearance of the formed lesions (indicated with yellow arrow) on the dissected muscle of the inner side of the right thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 16 overlapping lesions on the surface of the muscle was calculated to be 21 mm x 22 mm while its depth was 7 mm. Figure 16 demonstrates the vertical cross middle dissection of the necrotic area that was created on the right thigh by applying a 4 x 4 grid pattern.

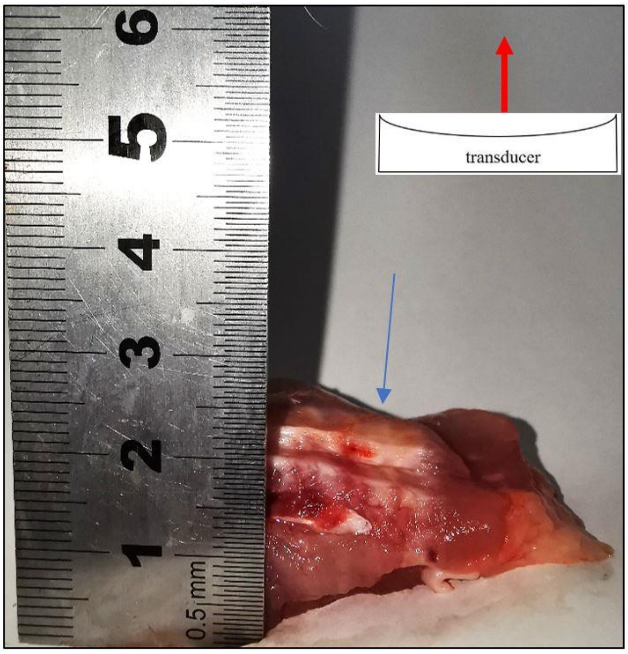

Figure 16: In situ dissection of the overlapping lesions on the right thigh. The blue arrow indicates the formed lesions and the red arrow indicates the beam direction.

Table 3 lists the anesthesia record for the rabbit experiment.

Table 3: Anesthesia record for rabbit 12.

Animal ID	SOUNDPET_2020_12				
Species	Rabbit				
Sex	F				
Age	Adult				
Weight (Kg)	2				
Supplier	S. Ioannou Farm (CYCU5.05)				
Grant	SOUNDPET (INTEGRATED/0918/0008)				
Study	CY/EXP/PR.L01/2020				
Participants	Kyriakos Spanoudes				
	Theocharis Drakos				
	Nikolas Evripidou				
Principal Investigator	Christakis Damianou				
Time	**Anesthesia**				
11:40	Medetomidine 1 ml + Ketamine 0.3 ml				
12:20	Medetomidine 0.5 ml + Ketamine 0.1 ml				
Euthanasia	12:35 1 ml T-61				
Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
11:40	110	52	0	Absent	n/a
11:50	110	52	0	Absent	n/a
12:00	100	56	0	Absent	n/a
12:10	100	58	0	Absent	n/a
12:20	98	62	0	Absent	n/a
12:30	102	56	1	Absent	n/a

Rabbit 13

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	300
Acoustic power (W)	93
Sonication time (s)	5
Energy (J)	465
Cooling time (s)	90
Number of sonications	16
Step between sonications (mm)	4
Distance transducer/rabbit skin (cm)	8

A 4 x 4 grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 16 at step movement of 4 mm and the distance between the transducer and the opening sonicated window of the robotic device was 8 cm.

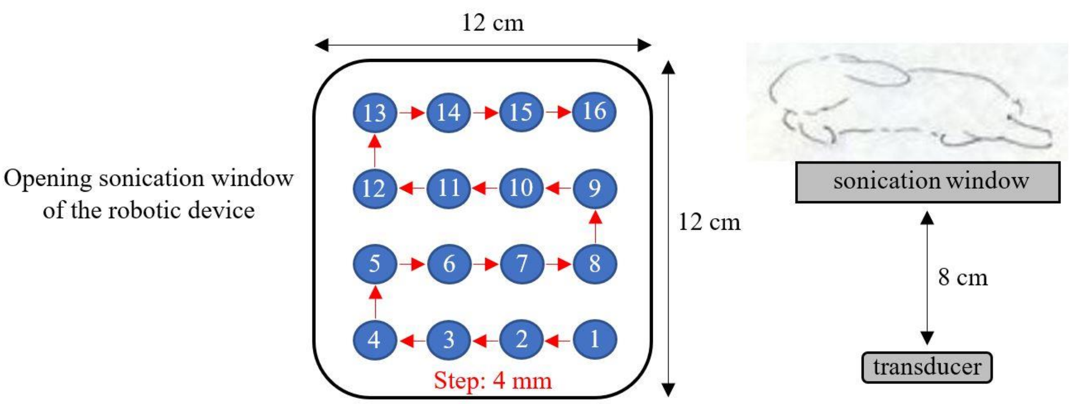

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	300
Acoustic power (W)	93
Sonication time (s)	3
Energy (J)	279
Cooling time (s)	90
Number of sonications	16
Step between sonications (mm)	4
Distance transducer/rabbit skin (cm)	7

A 4 x 4 grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved from point 1 to 16 at step movement of 4 mm and the distance between the transducer and the opening sonicated window of the robotic device was 8 cm.

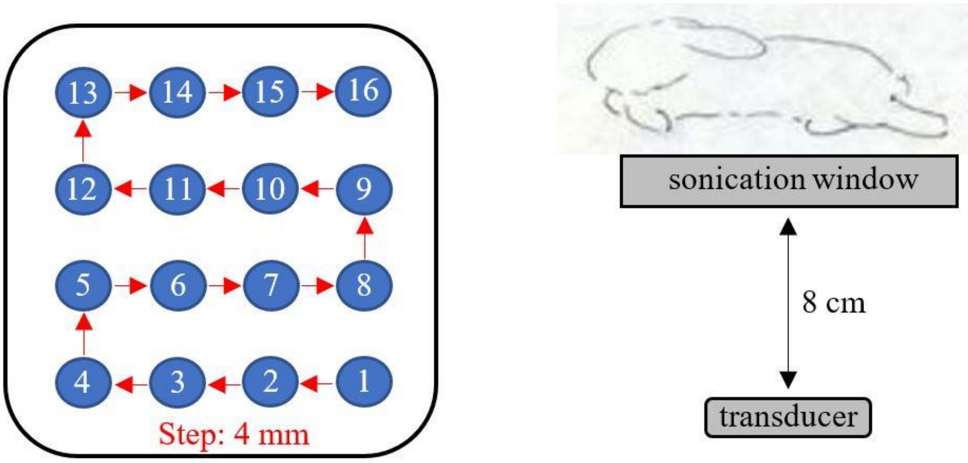

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory that was used for the rabbit thigh ablations.

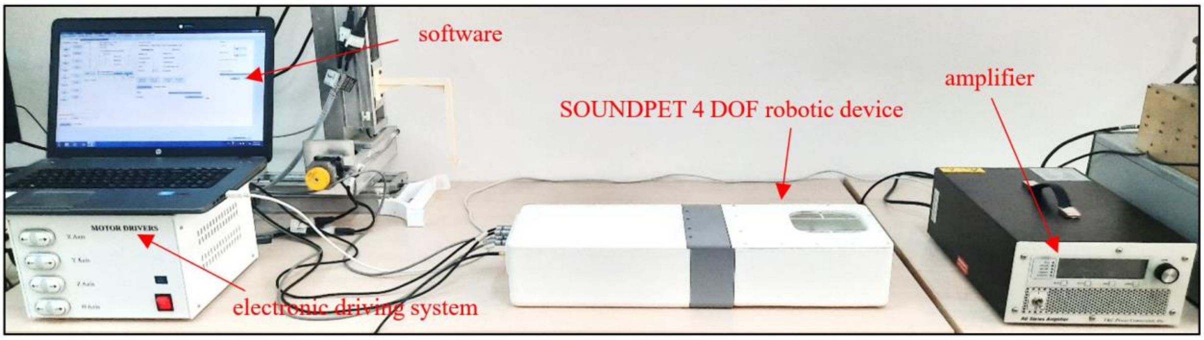

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

Figure 4: Animal positioning for the experiment.

Results

No lesions were observed from the side of the sonication (outer side) on the left thigh above and below the skin as shown in Figure 5 and Figure 6, respectively.

Figure 5: The outer side of the left thigh above the skin after the treatment.

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side).

Overlapping lesions were appeared on the inner side of the left thigh above and below the skin as shown in Figure 7 and Figure 8, respectively.

Figure 7: Macroscopic appearance of the overlapping lesions from the inner side of the left thigh and above the skin. The red arrow indicates the area of the created overlapping lesions on the skin.

Figure 8: Macroscopic appearance of the exposed muscle from the inner side of the left thigh. Overlapping lesions were formed after performing a 4 x 4 grid at step movement of 4 mm. The yellow arrow indicates the area of the created overlapping lesions on the exposed muscle.

The muscle of the left thigh was dissected and a necrotic region was observed as shown in Figure 9.

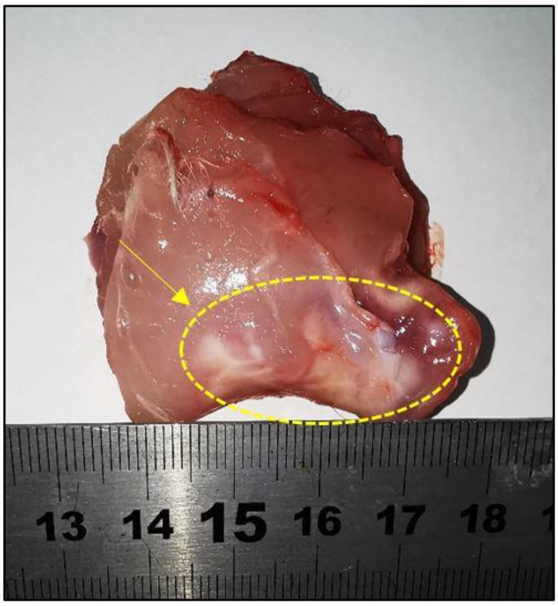

Figure 9: Macroscopic appearance of the overlapping lesions (indicated with yellow arrow) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 16 overlapping lesions on the surface of the muscle was calculated to be 26 mm x 11 mm while its depth was 11 mm. Figure 10 demonstrates the vertical cross middle dissection of the necrotic area that was created on the left thigh by applying a 4 x 4 grid pattern.

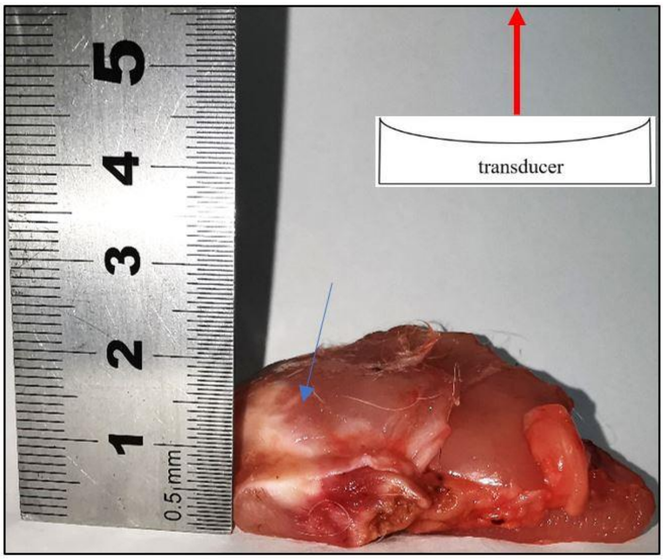

Figure 10: In situ dissection of the overlapping lesions that were created on the left thigh. The blue arrow indicates the necrotic area and the red arrow indicates the beam direction.

No lesions were observed from the side of the sonication (outer side) on the right thigh above and below the skin as shown in Figure 11 and Figure 12, respectively.

Figure 11: The outer side of the right thigh above the skin after the treatment.

Figure 12: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed.

Discrete lesions were appeared on the inner side of the right thigh above and below the skin as shown in Figure 13 and Figure 14, respectively.

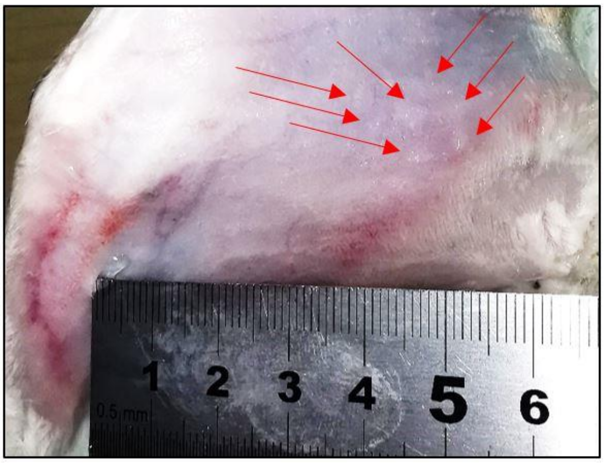

Figure 13: Macroscopic appearance of the overlapping lesions as observed from the inner side of the right thigh and above the skin. The red arrows indicate the discrete lesions that were observed on the skin.

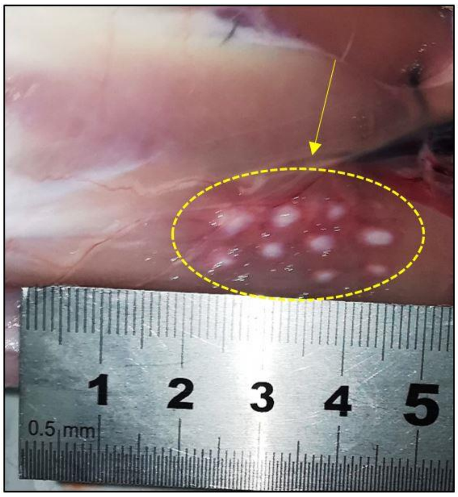

Figure 14: Macroscopic appearance of the discrete lesions on the exposed muscle from the inner side of the right thigh. The yellow arrow indicates the formed lesions after performing a 4 x 4 grid at step movement of 4 mm.

The muscle of the right thigh was dissected and ten discrete lesions were observed as shown in Figure 15.

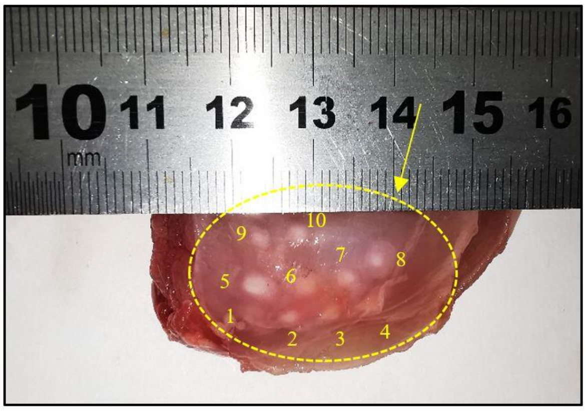

Figure 15: Macroscopic appearance of the formed lesions (indicated with yellow arrow) on the dissected muscle of the inner side of the right thigh (plane perpendicular to the beam).

The diameter of the formed lesions (ten lesions were visible after the muscle vertical dissection) was measured to be 3 mm, 2.5 mm, 2.5 mm, 4 mm, 5 mm, 4 mm, 2 mm, 4 mm, 3 mm, and 2.5 mm for lesion 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10, respectively. The average diameter of the formed discrete lesions was 3.6 mm. The depth of the lesion 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 was 4 mm, 10 mm, 12 mm, 8 mm, 11.5 mm, 9 mm, 8 mm, 10 mm, 7 mm, and 6 mm, respectively. Figure 16, Figure 17, and Figure 18 demonstrates the dissection of the lesions of the first row (1, 2, 3, 4), second row (5, 6, 7, 8) and third row (9, 10) that were created on the right thigh, respectively.

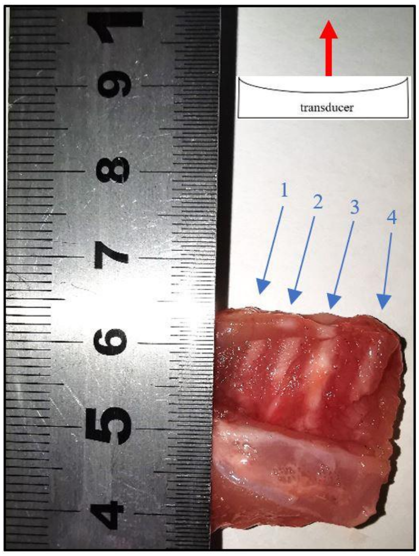

Figure 16: In situ dissection of the formed lesions of the first row (1, 2, 3, 4) of the right thigh. The blue arrows indicate the formed lesions and the red arrow indicates the beam direction.

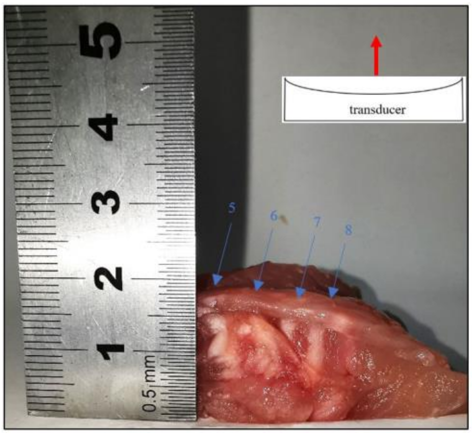

Figure 17: In situ dissection of the formed lesions of the second row (2, 3, 4, 5) of the right thigh. The blue arrows indicate the formed lesions and the red arrow indicates the beam direction.

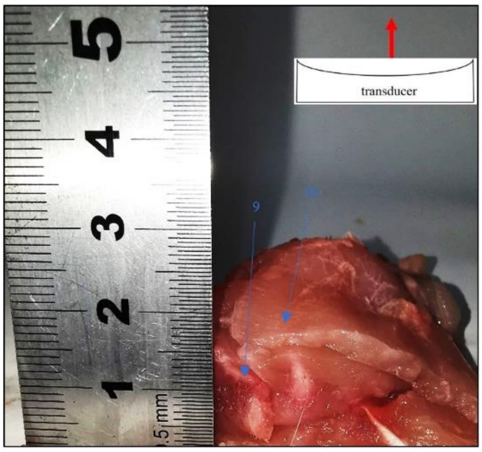

Figure 18: In situ dissection of the formed lesions of the third row (9, 10) of the right thigh. The blue arrows indicate the formed lesions and the red arrow indicates the beam direction.

Ten (10) lesions were observed on the right thigh after the treatment (Table 3). The diameter and length of the ten lesions was measurable.

Table 3: Summary of results for the right thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	3	4
2	2.5	10
3	2.5	12
4	4	8
5	5	11.5
6	4	9
7	2	8
8	4	10
9	3	7
10	2.5	6

Table 4 lists the anesthesia record for the rabbit experiment.

Table 4: Anesthesia record for rabbit 13.

Animal ID	SOUNDPET_2020_13
Species	Rabbit
Sex	F
Age	Adult
Weight (Kg)	2.02
Supplier	S. Ioannou Farm (CYCU5.05)
Grant	SOUNDPET (INTEGRATED/0918/0008)
Study	CY/EXP/PR.L01/2020
Participants	Kyriakos Spanoudes
	Theocharis Drakos
	Nikolas Evripidou
Principal Investigator	Christakis Damianou
Time	**Anesthesia**
09:35	Medetomidine 1 ml + Ketamine 0.3 ml
10:20	Medetomidine 0.5 ml + Ketamine 0.15 ml
Euthanasia	10:35 1 ml T-61

Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
09:40	100	44	0	Absent	n/a
09:50	110	48	0	Absent	n/a
10:00	112	44	0	Absent	n/a
10:10	110	48	0	Absent	n/a
10:20	108	52	0	Absent	n/a
10:30	104	52	1	Absent	n/a

Rabbit 14

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	300
Acoustic power (W)	93
Sonication time (s)	5
Energy (J)	465
Cooling time (s)	90
Number of sonications	25
Step between sonications (mm)	5
Distance transducer/rabbit skin (cm)	7

A 5 x 5 grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 25 at step movement of 5 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

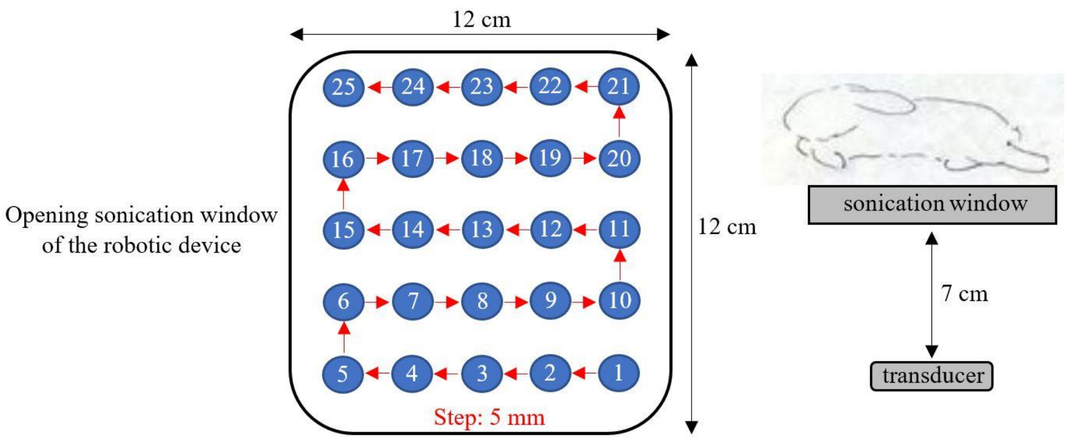

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	300
Acoustic power (W)	93
Sonication time (s)	5
Energy (J)	465
Cooling time (s)	90
Number of sonications	25
Step between sonications (mm)	5
Distance transducer/rabbit skin (cm)	7

A 5 x 5 grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved from point 1 to 25 at step movement of 5 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

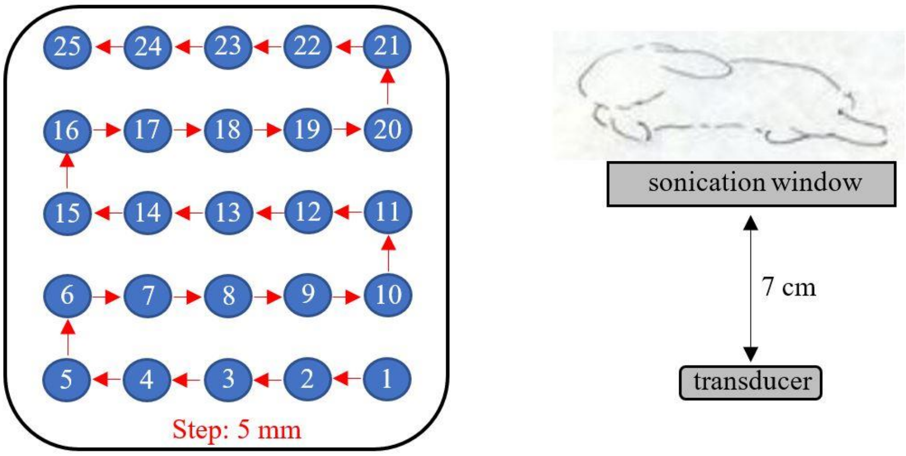

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory that was used for the rabbit thigh ablations.

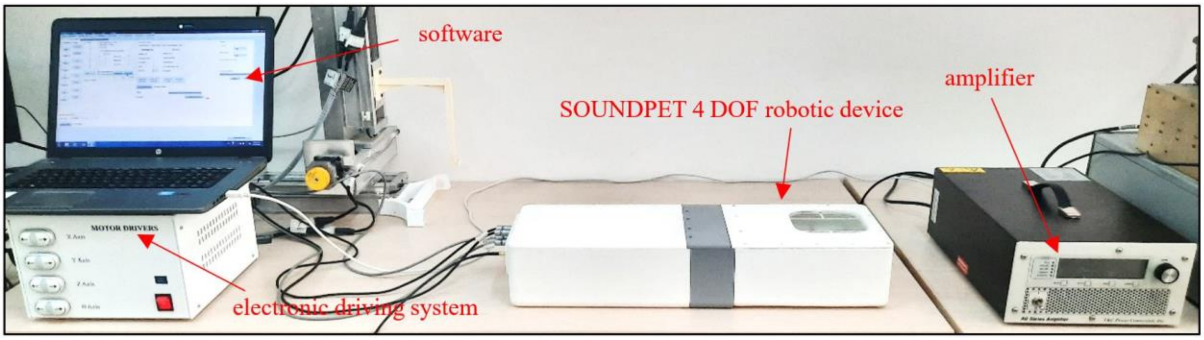

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

Figure 4: Animal positioning for the experiment.

Results

No lesions were observed from the side of the sonication (outer side) on the left thigh above and below the skin as shown in Figure 5 and Figure 6, respectively.

Figure 5: The outer side of the left thigh above the skin after the treatment.

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side).

Overlapping lesions were appeared on the inner side of the left thigh above and below the skin as shown in Figure 7 and Figure 8, respectively.

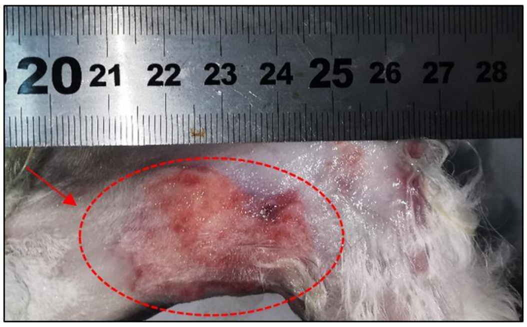

Figure 7: Macroscopic appearance of the overlapping lesions from the inner side of the left thigh and above the skin. The red arrow indicates the area of the created overlapping lesions on the skin.

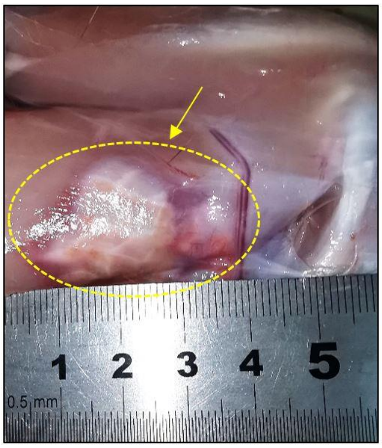

Figure 8: Macroscopic appearance of the exposed muscle from the inner side of the left thigh. Overlapping lesions were formed after performing a 5 x 5 grid at step movement of 5 mm. The yellow arrow indicates the area of the created overlapping lesions on the exposed muscle.

The muscle of the left thigh was dissected and a necrotic region was observed as shown in Figure 9.

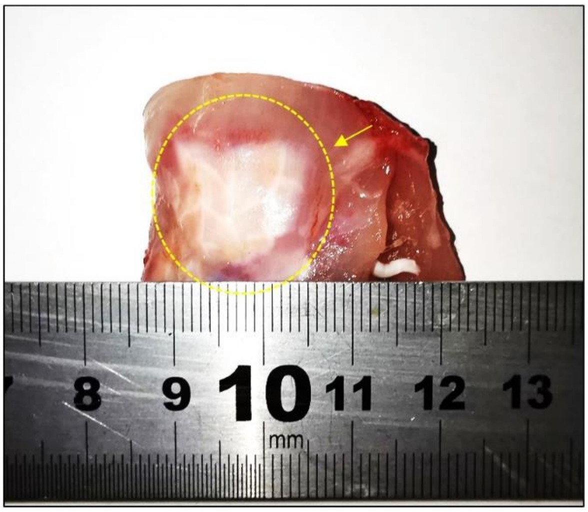

Figure 9: Macroscopic appearance of the overlapping lesions (indicated with yellow arrow) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 25 overlapping lesions on the surface of the muscle was calculated to be 15 mm x 14 mm while its depth was 7 mm. Figure 10 demonstrates the vertical cross middle dissection of the necrotic area that was created on the left thigh by applying a 5 x 5 grid pattern.

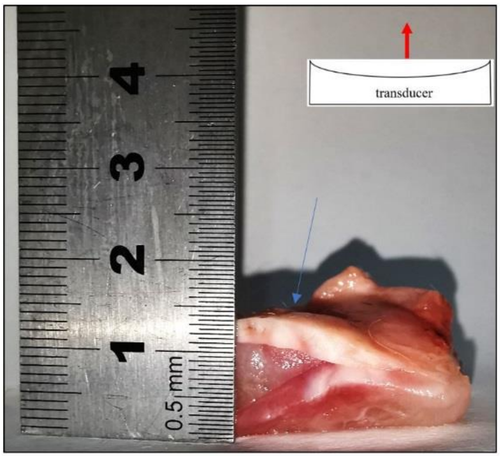

Figure 10: In situ dissection of the overlapping lesions that were created on the left thigh. The blue arrow indicates the necrotic area and the red arrow indicates the beam direction.

No lesions were observed from the side of the sonication (outer side) on the right thigh above and below the skin as shown in Figure 11 and Figure 12, respectively.

Figure 11: The outer side of the right thigh above the skin after the treatment.

Figure 12: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed.

Overlapping lesions were appeared on the inner side of the right thigh above and below the skin as shown in Figure 13 and Figure 14, respectively.

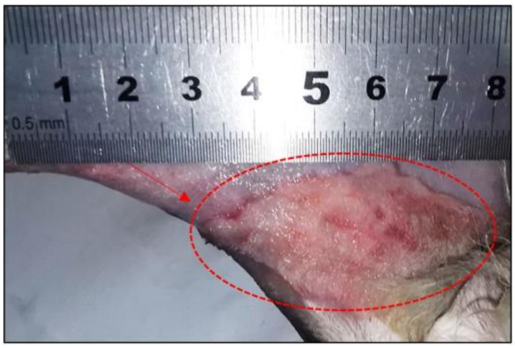

Figure 13: Macroscopic appearance of the overlapping lesions as observed from the inner side of the right thigh and above the skin. The red arrow indicates the area of the created overlapping lesions on the skin.

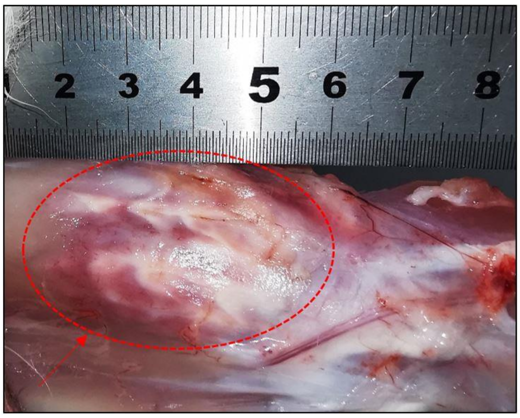

Figure 14: Macroscopic appearance of the overlapping lesions on the exposed muscle from the inner side of the right thigh. The red arrow indicates the formed lesions after performing a 5 x 5 grid at step movement of 5 mm.

The muscle of the right thigh was dissected and a necrotic region was observed as shown in Figure 15.

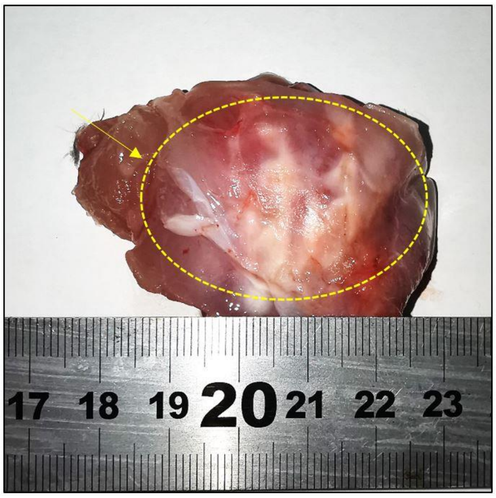

Figure 15: Macroscopic appearance of the formed lesions (indicated with yellow arrow) on the dissected muscle of the inner side of the right thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 25 overlapping lesions on the surface of the muscle was calculated to be 24 mm x 25 mm while its depth was 10 mm. Figure 16 demonstrates the vertical cross middle dissection of the necrotic area that was created on the right thigh by applying a 5 x 5 grid pattern.

Figure 16: In situ dissection of the overlapping lesions on the right thigh. The blue arrow indicates the formed lesions and the red arrow indicates the beam direction.

Table 3 lists the anesthesia record for the rabbit experiment.

Table 3: Anesthesia record for rabbit 14.

Animal ID	SOUNDPET_2020_14
Species	Rabbit
Sex	F
Age	Adult
Weight (Kg)	2.1
Supplier	S. Ioannou Farm (CYCU5.05)
Grant	SOUNDPET (INTEGRATED/0918/0008)
Study	CY/EXP/PR.L01/2020
Participants	Kyriakos Spanoudes
	Theocharis Drakos
	Nikolas Evripidou
Principal Investigator	Christakis Damianou
Time	**Anesthesia**
11:50	Medetomidine 1 ml + Ketamine 0.3 ml
12:30	Medetomidine 0.5 ml + Ketamine 0.15 ml
Euthanasia	12:40 1 ml T-61

Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
11:50	100	68	2	Absent	n/a

12:00	110	68	0	Absent	n/a
12:10	110	72	0	Absent	n/a
12:20	100	64	0	Absent	n/a
12:30	110	60	2	Absent	n/a
12:40	108	60	0	Absent	n/a
12:50	108	62	0	Absent	n/a
13:00	108	62	0	Absent	n/a

Rabbit 15

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	300
Acoustic power (W)	93
Sonication time (s)	5
Energy (J)	465
Cooling time (s)	90
Number of sonications	3
Step between sonications (mm)	10
Distance transducer/rabbit skin (cm)	7

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved reverse from point 1 to 3 at step movement of 10 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

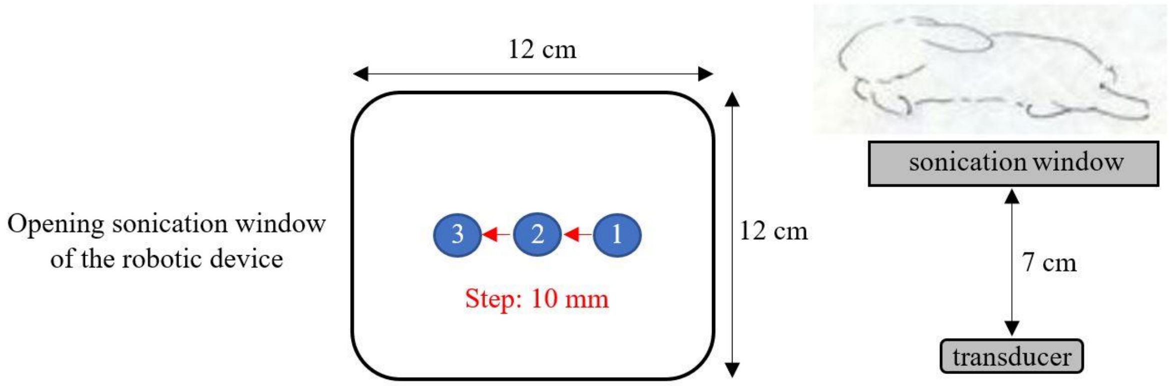

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	300
Acoustic power (W)	93
Sonication time (s)	10
Energy (J)	930
Cooling time (s)	90
Number of sonications	3
Step between sonications (mm)	10
Distance transducer/rabbit skin (cm)	7

A linear grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved reverse from point 1 to 3 at step movement of 10 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

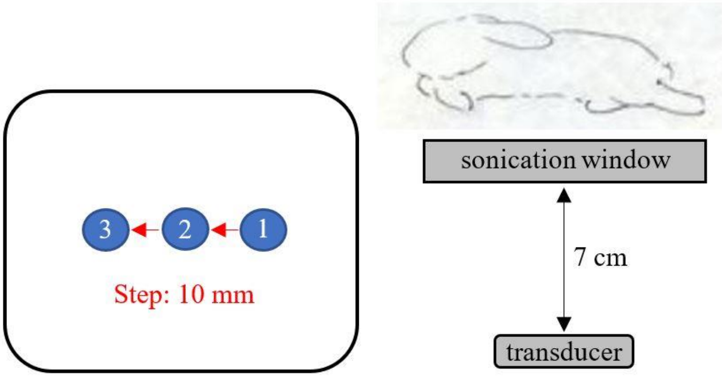

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory that was used for the rabbit thigh ablations.

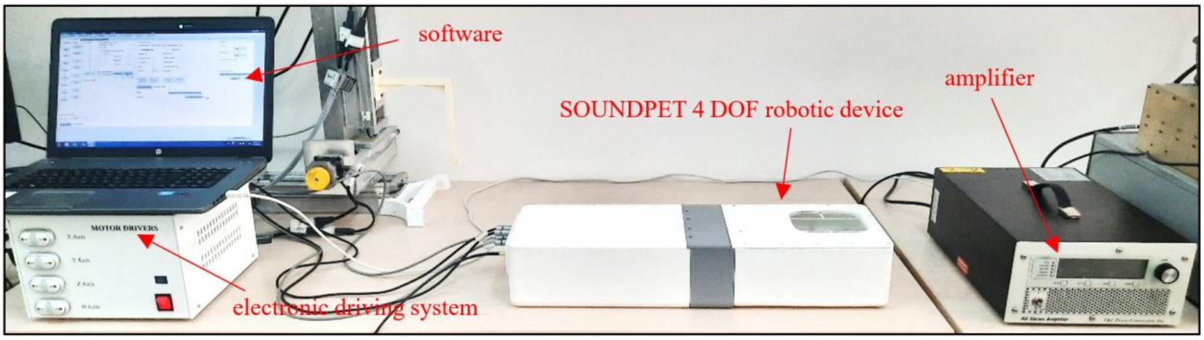

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

Figure 4: Animal positioning for the experiment.

Results

As shown in Figure 5 and Figure 6, no lesions were appeared from the side of the sonication (outer side) on the left thigh above and below the skin, respectively.

Figure 5: The outer side of the left thigh above the skin after the treatment.

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side).

Discrete lesions were observed on the inner side of the left thigh both above and below the skin as shown in Figure 7 and Figure 8, respectively.

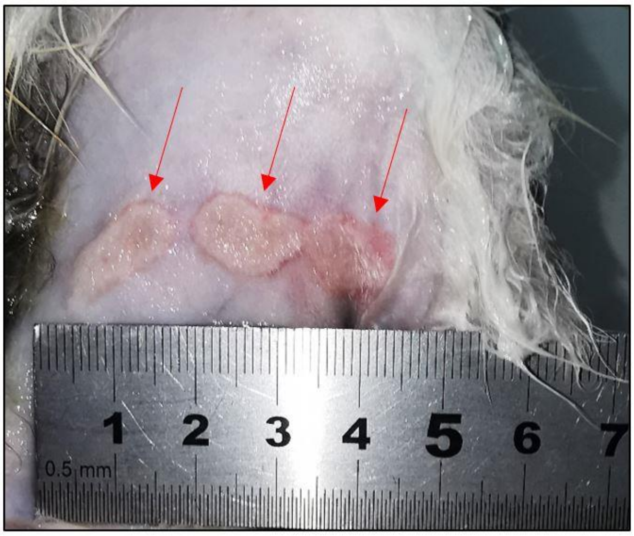

Figure 7: Macroscopic appearance of the lesions from the inner side of the left thigh and above the skin. The red arrows indicate the formed lesions on the skin.

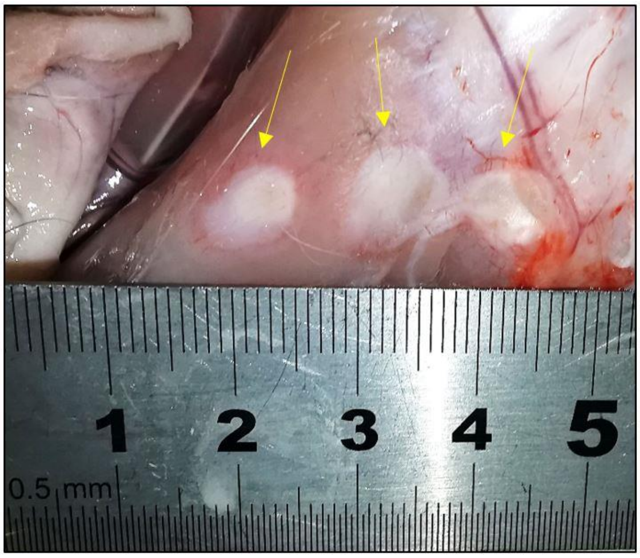

Figure 8: Macroscopic appearance of the discrete lesions on the exposed muscle from the inner side of the left thigh. The yellow arrows indicate the lesions after performing a linear grid of 3 sonications at step movement of 10 mm.

The muscle of the left thigh was dissected and three discrete lesions were observed as shown in Figure 9.

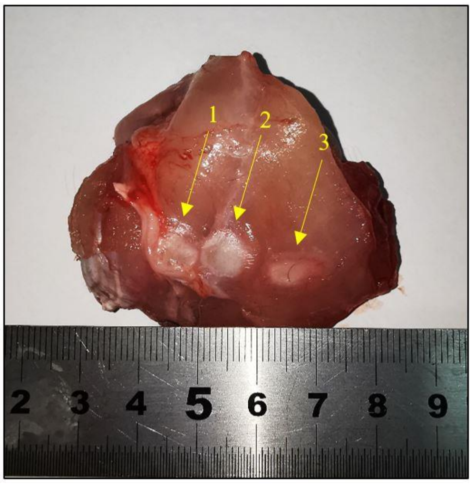

Figure 9: Macroscopic appearance of the formed lesions (indicated with yellow arrows) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The diameter of the formed lesions (three lesions were visible after the muscle vertical dissection) was measured to be 7 mm, 8 mm, and 9 mm for lesion 1, 2, and 3, respectively. The average diameter of the formed discrete lesions was 8 mm. The depth of the lesion 1, 2, and 3 was 8 mm, 4 mm, and 8 mm, respectively. Figure 10 and Figure 11 demonstrates the dissection of the lesions (2, 3) and lesion 1 that were created on the left thigh, respectively.

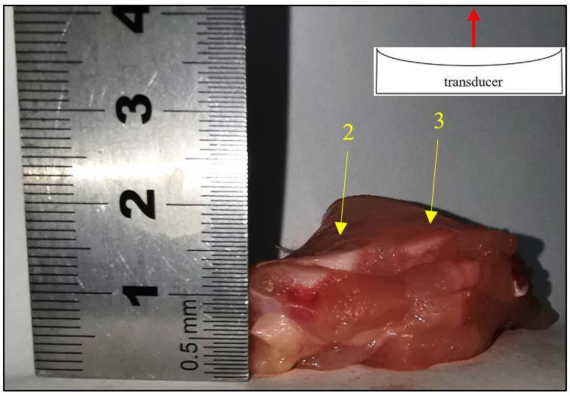

Figure 10: In situ dissection of the formed lesions 2 and 3 of the left thigh. The yellow arrows indicate the formed lesions and the red arrow indicates the beam direction.

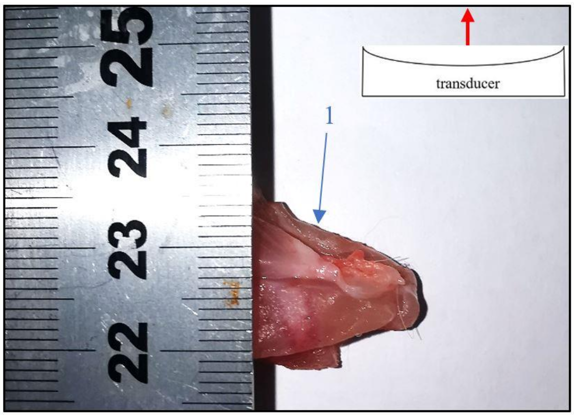

Figure 11: In situ dissection of the formed lesion 1 of the left thigh. The blue arrow indicates the formed lesion and the red arrow indicates the beam direction.

Three (3) lesions were observed on the left thigh after the treatment (Table 3). The diameter and length of the three lesions was measurable.

Table 3: Summary of results for the left thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	7	8
2	8	4
3	9	8

No lesions were observed from the side of the sonication (outer side) on the right thigh above and below the skin as shown in Figure 12 and Figure 13, respectively.

Figure 12: The outer side of the right thigh above the skin after the treatment.

Figure 13: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed.

The lesions were appeared to overlap on the inner side of the right thigh above the skin as shown in Figure 14 and partially discrete below the skin as shown in Figure 15.

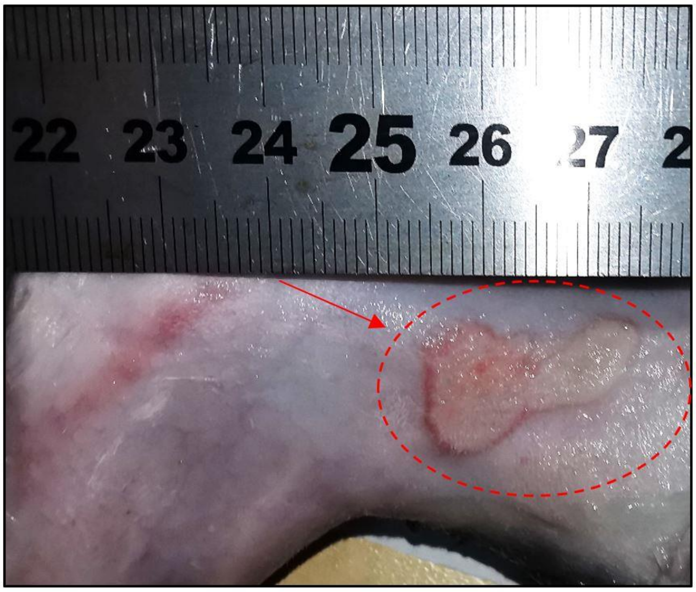

Figure 14: Macroscopic appearance of the overlapping lesions as observed from the inner side of the right thigh and above the skin. The red arrow indicates the overlapping lesions that were observed on the skin.

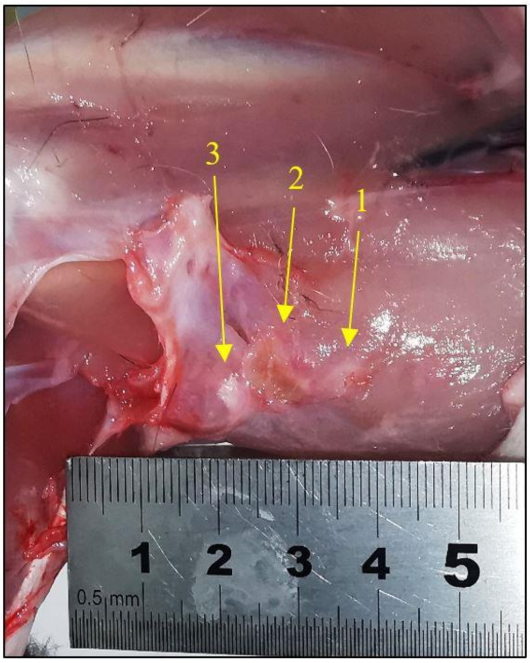

Figure 15: Macroscopic appearance of the discrete lesions on the exposed muscle from the inner side of the right thigh. The yellow arrows indicate the formed lesions after performing a 1 x 3 grid at step movement of 10 mm.

The muscle of the right thigh was dissected and two overlapping lesions and one discrete lesion were observed as shown in Figure 16.

Figure 16: Macroscopic appearance of the formed lesions (indicated with yellow arrows) on the dissected muscle of the inner side of the right thigh (plane perpendicular to the beam).

Figure 17 demonstrates the vertical cross middle dissection of the lesions that were created on the right thigh.

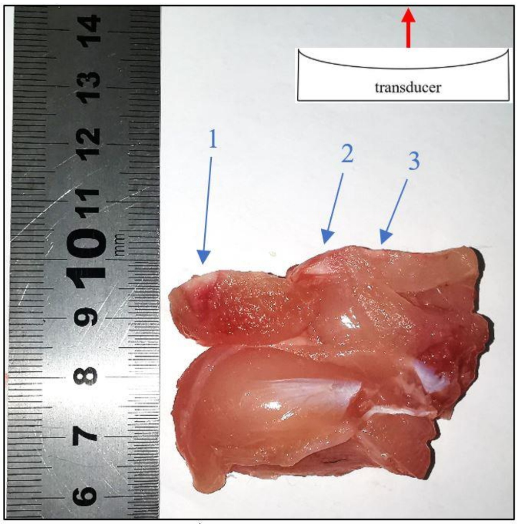

Figure 17: In situ dissection of the formed lesions of the right thigh. The blue arrows indicate the formed lesions and the red arrow indicates the beam direction.

Three (3) lesions were observed on the right thigh after the treatment (Table 4). The diameter and length of the three lesions was measurable.

Table 4: Summary of results for the right thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	8	8
2	4	4
3	4.5	2

Table 5 lists the anesthesia record for the rabbit experiment.

Table 5: Anesthesia record for rabbit 15.

Animal ID	SOUNDPET_2020_15				
Species	Rabbit				
Sex	M				
Age	Adult				
Weight (Kg)	2.15				
Supplier	S. Ioannou Farm (CYCU5.05)				
Grant	SOUNDPET (INTEGRATED/0918/0008)				
Study	CY/EXP/PR.L01/2020				
Participants	Kyriakos Spanoudes				
	Theocharis Drakos				
	Nikolas Evripidou				
Principal Investigator	Christakis Damianou				
Time	**Anesthesia**				
10:15	Medetomidine 1 ml + Ketamine 0.3 ml				
10:20	Medetomidine 0.3 ml				
Euthanasia	10:30 1 ml T-61				
Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
10:15	110	62	1	Absent	n/a
10:20	100	66	0	Absent	n/a
10:25	106	66	0	Absent	n/a
10:30	106	64	0	Absent	n/a

Radiography

An x-ray was taken prior treatment with an X-ray system (IMS001, Shenzhen Browiner Tech Co., Ltd). The animal was placed above a CR (Computed Radiography) cassette. The digital image was developed using a CR reader (Vita Flex, Carestream Health, Inc., 150 Verona Street, Rochester, NY, USA). Prior treatment, the purpose of X-ray was to calculate the available muscle area for ablation, thus avoiding bone. The findings of the x-ray taken from the animal, prior treatments, can be seen in the following Figure 18 which demonstrates radiography of the rabbit thigh. This view allowed demarcation of the available for ablation area and decision-making regarding the number of the lesions.

Figure 18: X-ray taken prior treatment.

Ultrasound Imaging

Prior and following treatment, ultrasound examination of the thigh was performed using a portable ultrasound device (Mindray DP 50, Shenzhen, China) with a linear probe. Figure 19 and Figure 20 shows the ultrasound image of the rabbit muscle taken prior and following treatment, respectively.

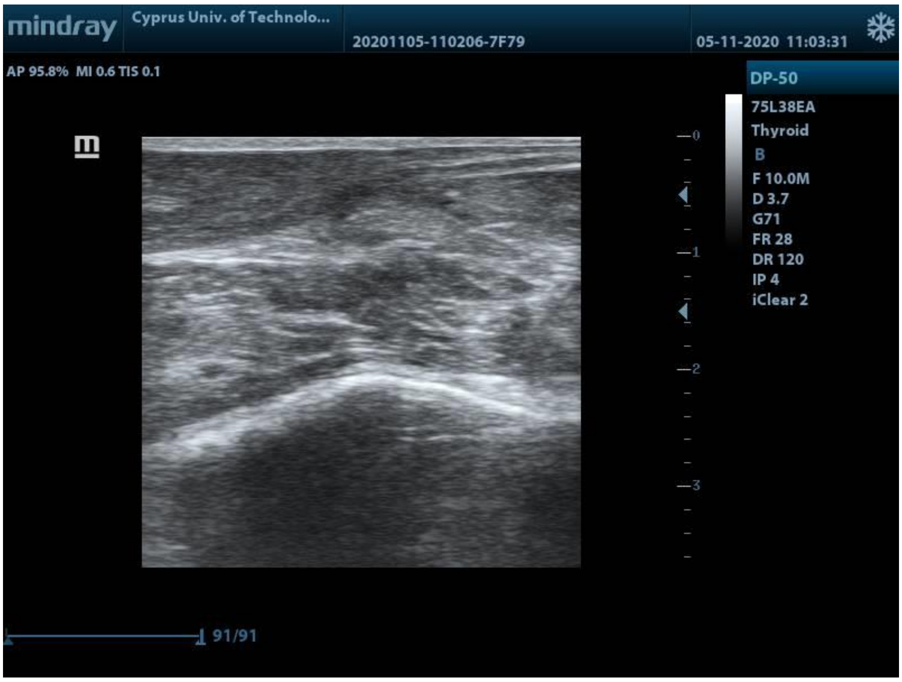

Figure 19: Ultrasound capture of the rabbit muscle prior treatment.

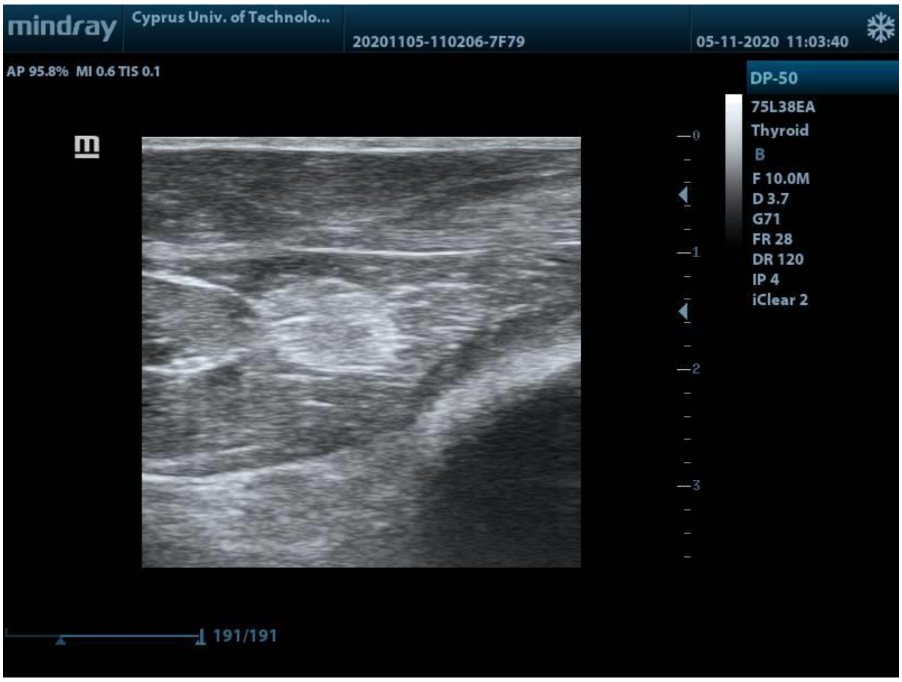

Figure 20: Ultrasound capture of the rabbit muscle following treatment.

Rabbit 16

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	300
Acoustic power (W)	93
Sonication time (s)	10
Energy (J)	930
Cooling time (s)	90
Number of sonications	3
Step between sonications (mm)	10
Distance transducer/rabbit skin (cm)	7

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved reverse from point 1 to 3 at step movement of 10 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

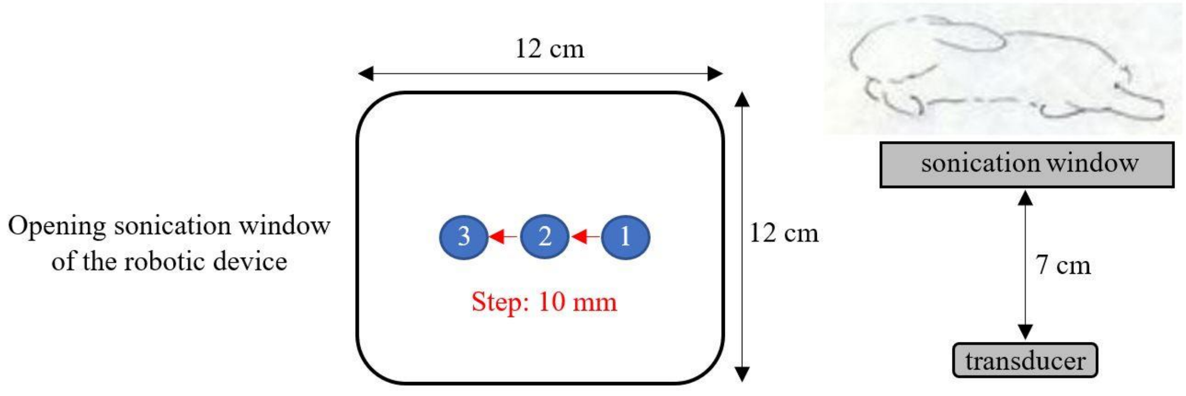

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	300
Acoustic power (W)	93
Sonication time (s)	5
Energy (J)	465
Cooling time (s)	90
Number of sonications	3
Step between sonications (mm)	10
Distance transducer/rabbit skin (cm)	7

A linear grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved reverse from point 1 to 3 at step movement of 10 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

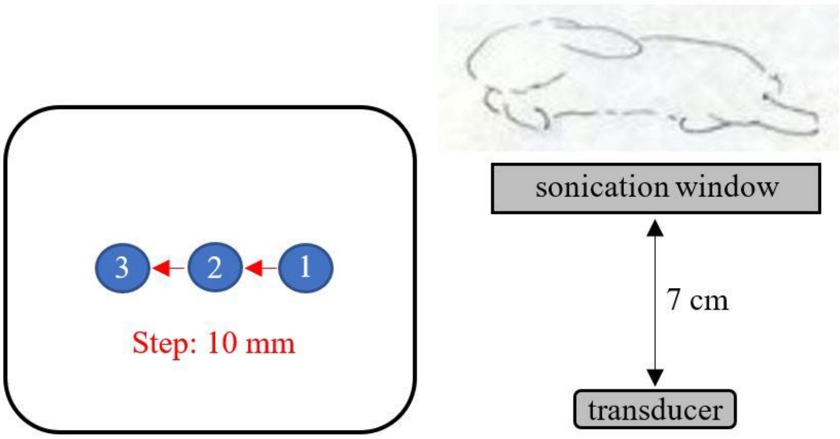

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory, that was used for the rabbit thigh ablations.

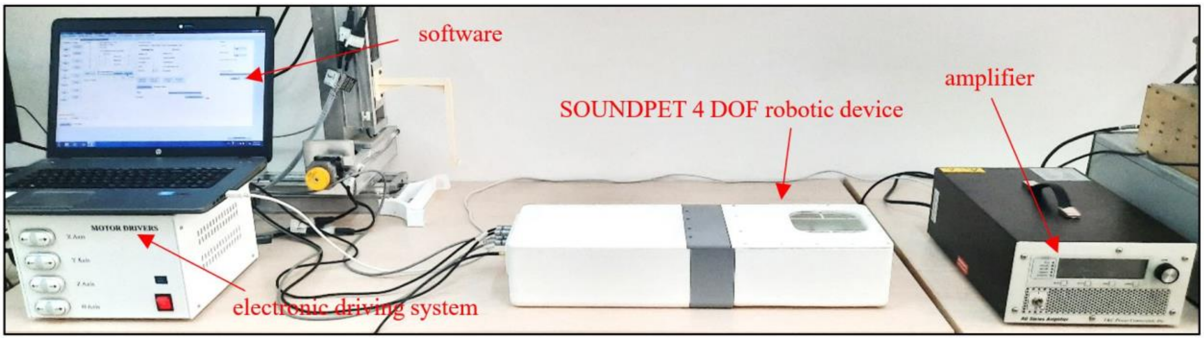

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

Figure 4: Animal positioning for the experiment.

Results

As shown in Figure 5 and Figure 6, no lesions were appeared on the outer side (sonication side) of the left thigh above and below the skin, respectively.

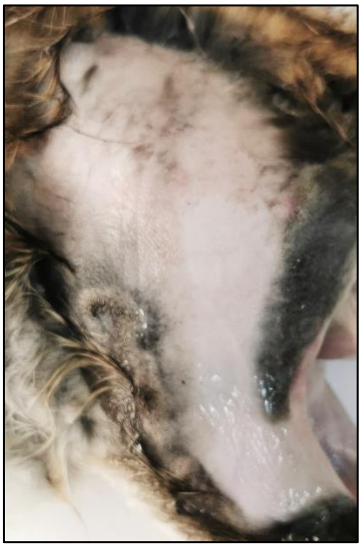

Figure 5: The outer side of the left thigh above the skin after the treatment.

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side).

Discrete lesions were observed on the inner side of the left thigh above the skin as shown in Figure 7. A single faint lesion was observed on the inner side of the left thigh above the skin as shown in Figure 8.

Figure 7: Macroscopic appearance of the discrete lesions from the inner side of the left thigh and above the skin. The red arrows indicate the lesions on the skin.

Figure 8: Macroscopic appearance of the formed lesion on the exposed muscle from the inner side of the left thigh. The red arrow indicates the only formed lesion after performing a linear grid of 3 sonications at step movement of 10 mm.

The muscle of the left thigh was dissected and only one faint lesion (4 mm diameter) was appeared as shown in Figure 9.

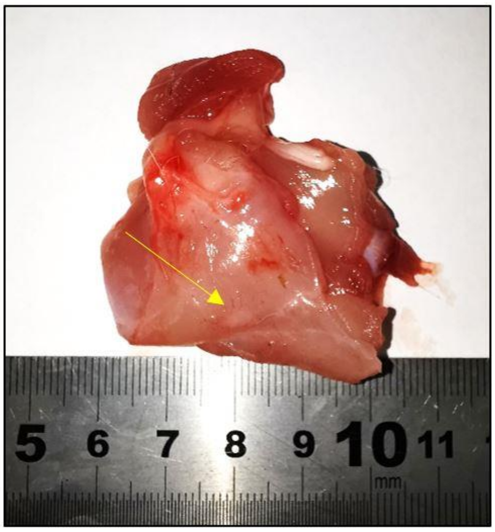

Figure 9: Macroscopic appearance of the formed lesion (indicated with yellow arrow) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam). The lesion was superficial.

As shown in Figure 10 and Figure 11, no lesions were appeared on the right thigh above and below the skin from the side of the sonication, respectively.

Figure 10: The right thigh following treatment above the skin from the side of the sonication.

Figure 11: The right thigh following treatment after the skin was removed and the muscle was exposed from the side of the sonication.

Discrete lesions were formed on the inner side of the right thigh both above and below the skin as shown in Figure 12 and Figure 13, respectively.

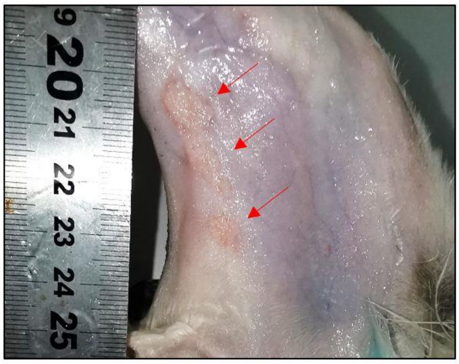

Figure 12: Macroscopic appearance of the lesions from the inner side of the right thigh and above the skin. The red arrows indicate the discrete lesions that were appeared on the skin.

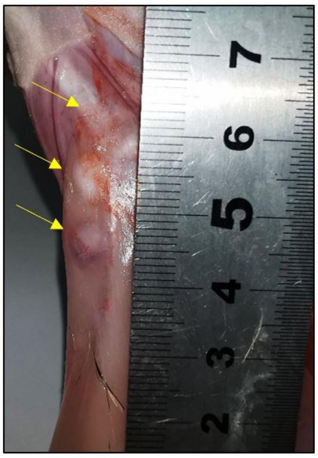

Figure 13: Macroscopic appearance of the discrete lesions on the exposed muscle from the inner side of the right thigh. The yellow arrows indicate the formed lesions after performing a linear grid of 3 sonications at step movement of 10 mm.

The muscle of the inner side of the right thigh was dissected and three (3) discrete lesions were appeared as shown in Figure 14.

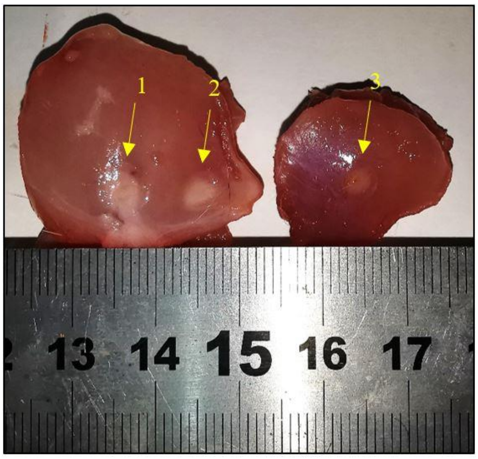

Figure 14: Macroscopic appearance of the formed lesions (indicated with yellow arrows) on the dissected muscle of the inner side of the right thigh (plane perpendicular to the beam).

The diameter of the formed lesions (three lesions were visible after the muscle vertical dissection) was measured to be 6 mm, 4 mm, and 3 mm for lesion 1, 2, and 3, respectively. The average diameter of the formed discrete lesions was 4.3 mm. The depth of the lesion 1, 2, and 3 was 2 mm, 5 mm, and 3 mm, respectively. Figure 15 and Figure 16 demonstrates the dissection of the lesions (1, 2) and lesion 3 that were created on the right thigh, respectively.

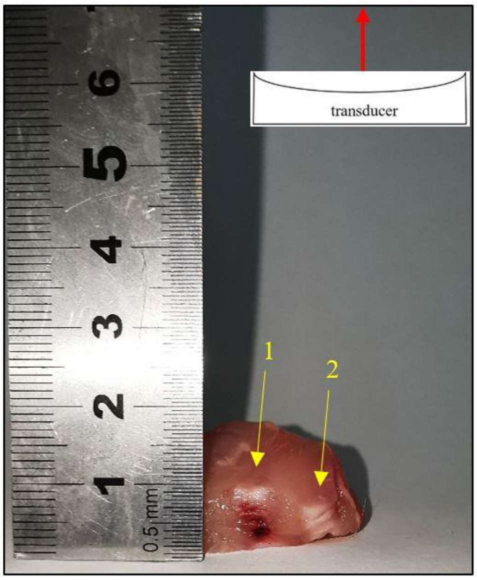

Figure 15: In situ dissection of the formed lesions 1 and 2 of the right thigh. The yellow arrows indicate the formed lesions and the red arrow indicates the beam direction.

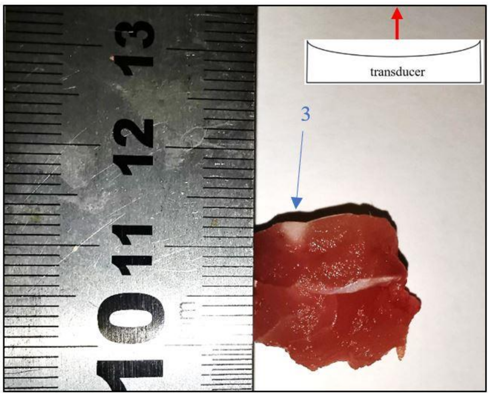

Figure 16: In situ dissection of the formed lesion 3 of the right thigh. The blue arrow indicates the formed lesions and the red arrow indicates the beam direction.

Three (3) lesions were observed on the right thigh after the treatment (Table 3). The diameter and length of the three lesions was measurable.

Table 3: Summary of results for the right thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	6	2
2	4	5
3	3	3

Table 4 lists the anesthesia record for the rabbit experiment.

Table 4: Anesthesia record for rabbit 16.

Animal ID	SOUNDPET_2020_16
Species	Rabbit
Sex	M
Age	Adult
Weight (Kg)	2.2
Supplier	S. Ioannou Farm (CYCU5.05)
Grant	SOUNDPET (INTEGRATED/0918/0008)
Study	CY/EXP/PR.L01/2020
Participants	Kyriakos Spanoudes
	Theocharis Drakos
	Nikolas Evripidou
Principal Investigator	Christakis Damianou
Time	**Anesthesia**
11:50	Medetomidine 1 ml + Ketamine 0.3 ml
12:00	Medetomidine 0.3 ml
Euthanasia	12:20 1 ml T-61

Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
11:50	100	64	2	Absent	n/a
12:00	100	62	0	Absent	n/a
12:10	98	68	0	Absent	n/a
12:20	102	72	0	Absent	n/a

Rabbit 17

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	350
Acoustic power (W)	108.5
Sonication time (s)	5
Energy (J)	542.5
Cooling time (s)	90
Number of sonications	3
Step between sonications (mm)	10
Distance transducer/rabbit skin (cm)	7

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved reverse from point 1 to 3 at step movement of 10 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

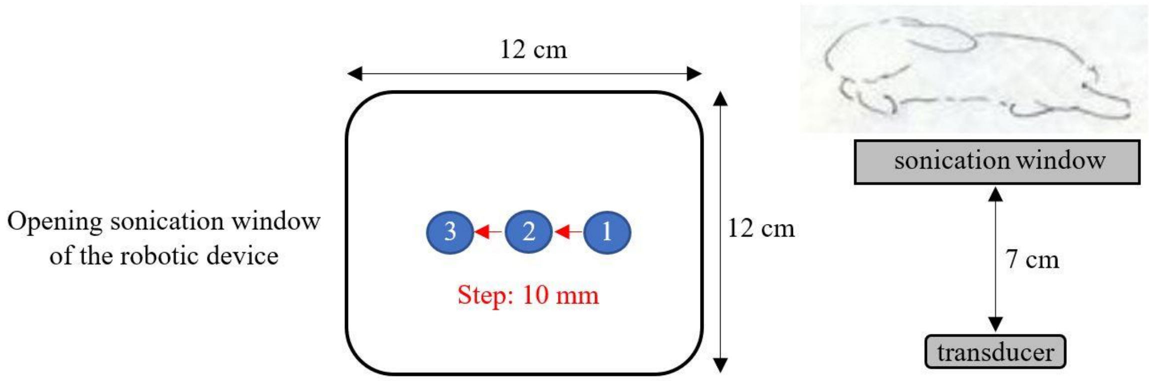

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	350
Acoustic power (W)	108.5
Sonication time (s)	3
Energy (J)	325.5
Cooling time (s)	90
Number of sonications	3
Step between sonications (mm)	10
Distance transducer/rabbit skin (cm)	7

A linear grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved reverse from point 1 to 3 at step movement of 10 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

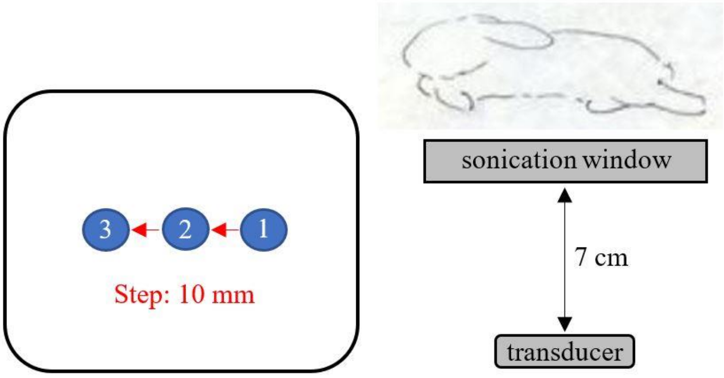

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory that was used for the rabbit thigh ablations.

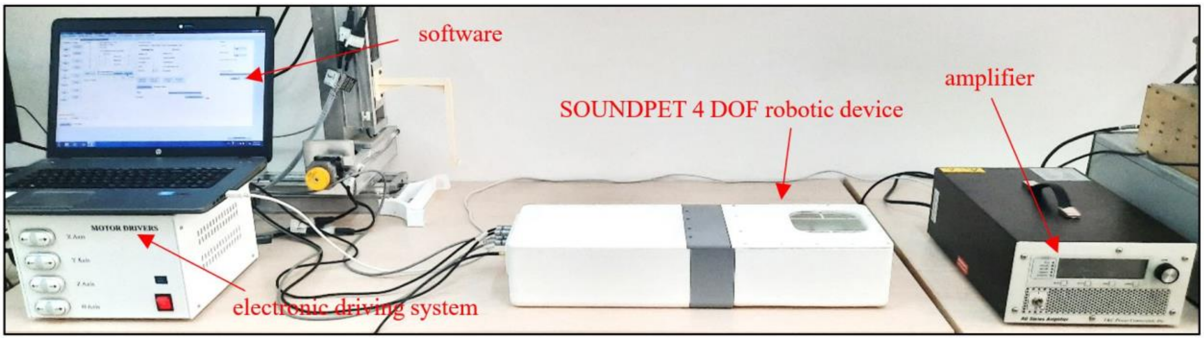

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

Figure 4: Animal positioning for the experiment.

Results

As shown in Figure 5 and Figure 6, no lesions were appeared from the side of the sonication (outer side) on the left thigh above and below the skin, respectively.

Figure 5: The outer side of the left thigh above the skin after the treatment.

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side).

Overlapping lesions were observed on the inner side of the left thigh above the skin as shown in Figure 7. However, discrete lesions were observed below the skin as shown in Figure 8.

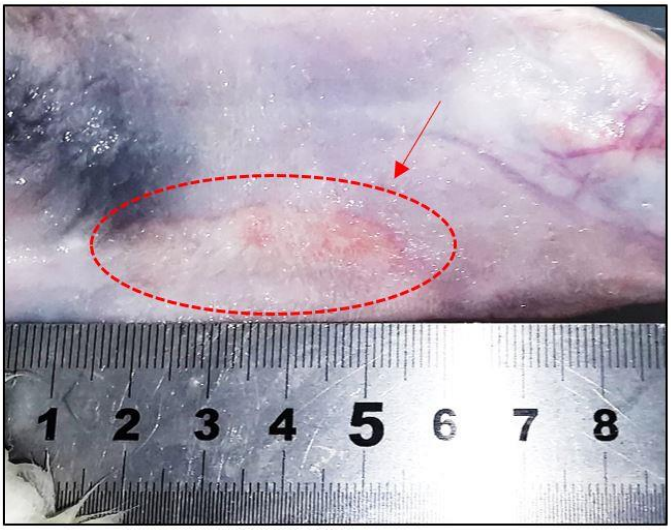

Figure 7: Macroscopic appearance of the lesions from the inner side of the left thigh and above the skin. The red arrow indicates the overlapping lesions on the skin.

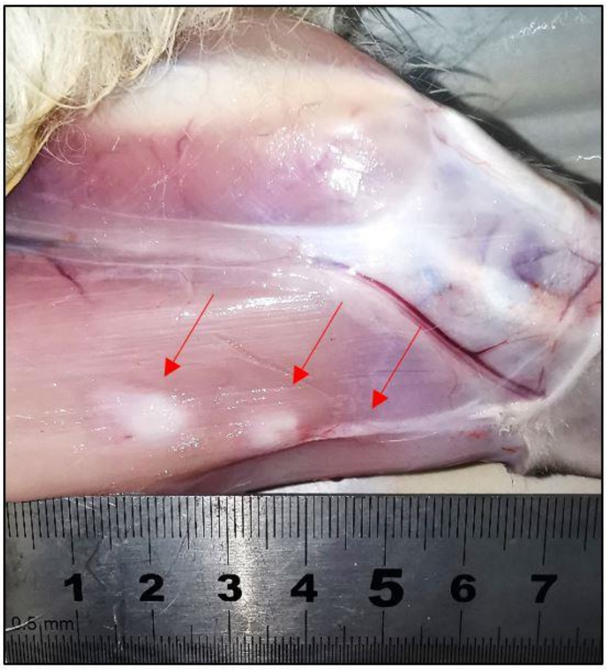

Figure 8: Macroscopic appearance of the discrete lesions on the exposed muscle from the inner side of the left thigh. The red arrows indicate the lesions after performing a linear grid of 3 sonications at step movement of 10 mm.

The muscle of the left thigh was dissected and three discrete lesions were observed as shown in Figure 9.

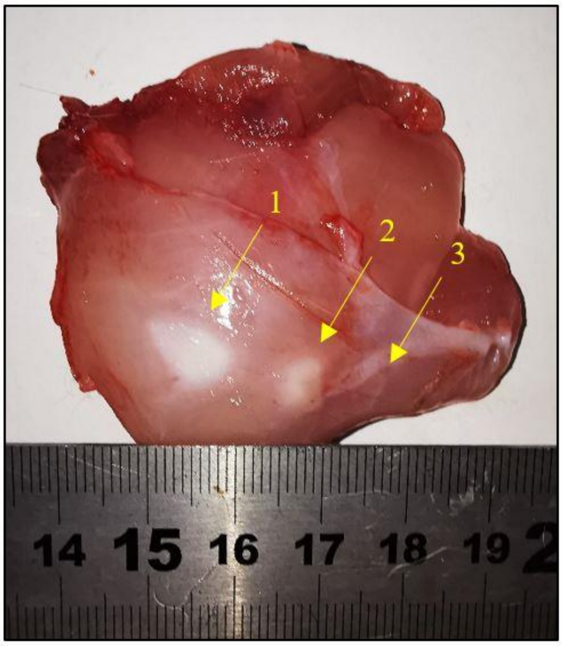

Figure 9: Macroscopic appearance of the formed lesions (indicated with yellow arrows) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The diameter of the formed lesions (three lesions were visible after the muscle exposure) was measured to be 10 mm, 6 mm, and 3 mm for lesion 1, 2, and 3, respectively. The average diameter of the formed discrete lesions was 6.3 mm. The depth of the lesion 1 and 2 was 3 mm. Figure 10 demonstrates the dissection of the lesions 1 and 2 that were created on the left thigh. Lesion 3 was superficial.

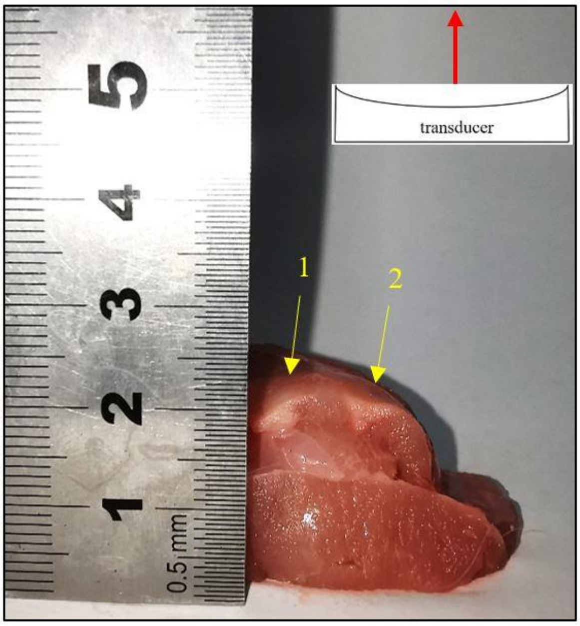

Figure 10: In situ dissection of the formed lesions 1 and 2 of the left thigh. The yellow arrows indicate the formed lesions and the red arrow indicates the beam direction.

Three (3) lesions were observed on the left thigh after the treatment (Table 3). The diameter of the three lesions was measurable while the length of the two was measurable since the one lesion was superficial.

Table 3: Summary of results for the left thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	10	3
2	6	3
3	3	-

No lesions were observed from the side of the sonication (outer side) on the right thigh above and below the skin as shown in Figure 11 and Figure 12, respectively.

Figure 11: The outer side of the right thigh above the skin after the treatment.

Figure 12: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed.

Overlapping lesions were observed on the inner side of the right thigh above the skin as shown in Figure 13. However, discrete lesions were observed below the skin as shown in Figure 14.

Figure 13: Macroscopic appearance of the overlapping lesions as observed from the inner side of the right thigh and above the skin. The red arrow indicates the overlapping lesions that were observed on the skin.

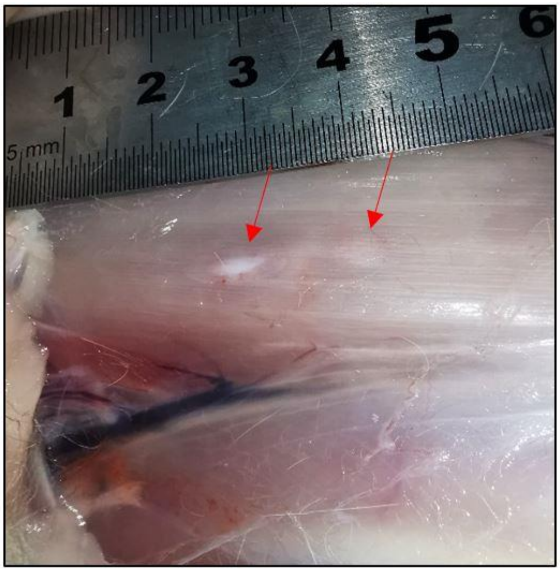

Figure 14: Macroscopic appearance of the discrete lesions on the exposed muscle from the inner side of the right thigh. The red arrows indicate the formed lesions after performing a 1 x 3 grid at step movement of 10 mm.

The muscle of the right thigh was dissected and two discrete lesions were observed as shown in Figure 15.

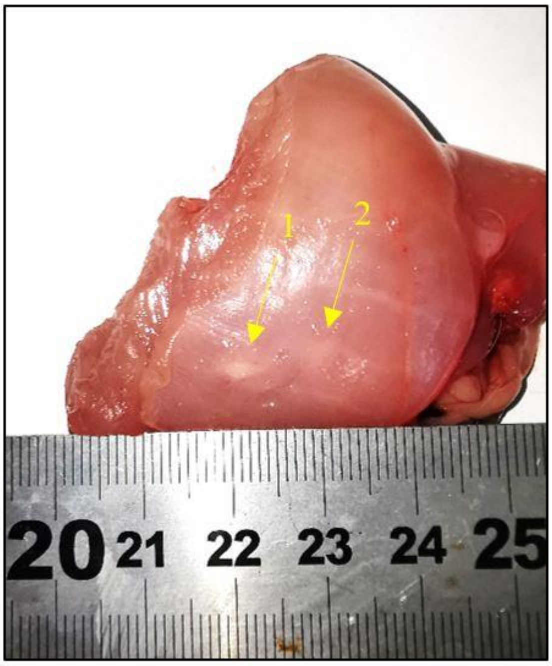

Figure 15: Macroscopic appearance of the formed lesions (indicated with yellow arrows) on the dissected muscle of the inner side of the right thigh (plane perpendicular to the beam).

Figure 16 demonstrates the vertical cross middle dissection of lesion 1 that was created on the right thigh. Lesion 2 was superficial.

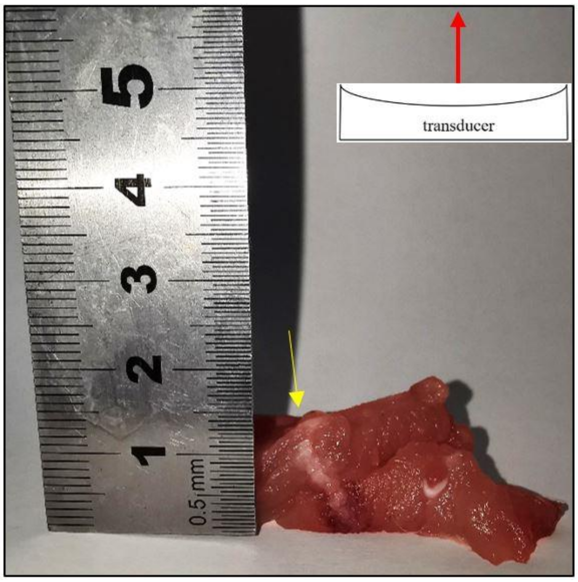

Figure 16: In situ dissection of the formed lesion 1 of the right thigh. The yellow arrow indicates the formed lesion and the red arrow indicates the beam direction.

Two (2) lesions were observed on the right thigh after the treatment (Table 4). The diameter and length of lesion 1 was measurable while the diameter of lesion 2 was only measurable since it was a superficial lesion.

Table 4: Summary of results for the right thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	4	10
2	4	-
3	-	-

Table 5 lists the anesthesia record for the rabbit experiment.

Table 5: Anesthesia record for rabbit 17.

Animal ID	SOUNDPET_2020_17				
Species	Rabbit				
Sex	F				
Age	Adult				
Weight (Kg)	2.2				
Supplier	S. Ioannou Farm (CYCU5.05)				
Grant	SOUNDPET (INTEGRATED/0918/0008)				
Study	CY/EXP/PR.L01/2020				
Participants	Kyriakos Spanoudes				
	Theocharis Drakos				
	Nikolas Evripidou				
Principal Investigator	Christakis Damianou				
Time	**Anesthesia**				
09:35	Medetomidine 1.5 ml + Ketamine 0.5 ml				
09:45	Medetomidine 0.5 ml				
Euthanasia	10:15 1 ml T-61				
Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
09:40	100	60	0	Absent	n/a
09:50	110	64	0	Absent	n/a
10:00	100	64	0	Absent	n/a
10:10	100	60	0	Absent	n/a

Rabbit 18

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	350
Acoustic power (W)	108.5
Sonication time (s)	5
Energy (J)	542.5
Cooling time (s)	90
Number of sonications	16
Step between sonications (mm)	5
Distance transducer/rabbit skin (cm)	7

A 4 x 4 grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 16 at step movement of 5 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

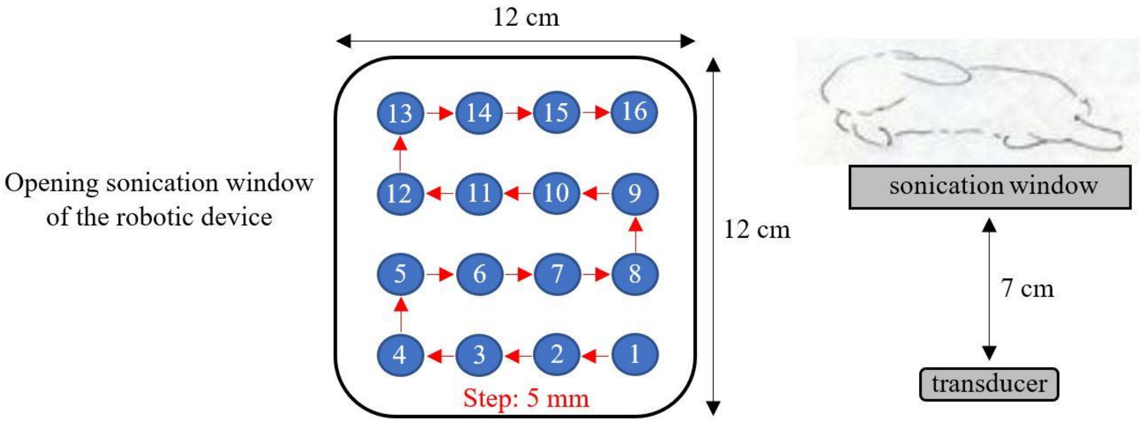

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	350
Acoustic power (W)	108.5
Sonication time (s)	3
Energy (J)	325.5
Cooling time (s)	90
Number of sonications	16
Step between sonications (mm)	5
Distance transducer/rabbit skin (cm)	7

A 4 x 4 grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved from point 1 to 16 at step movement of 5 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

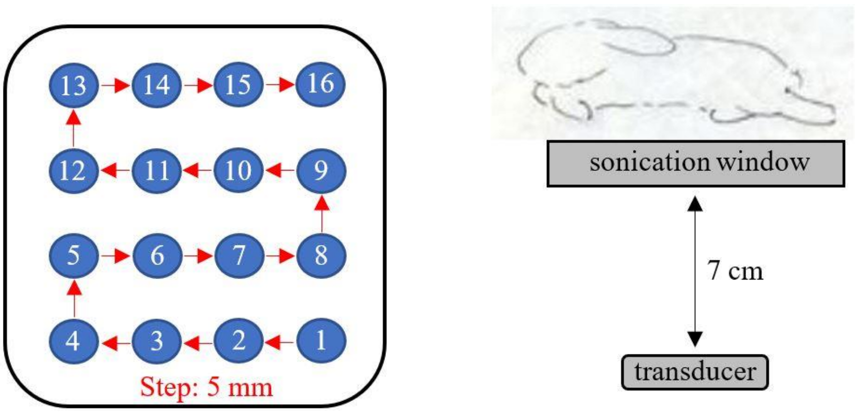

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory, that was used for the rabbit thigh ablations.

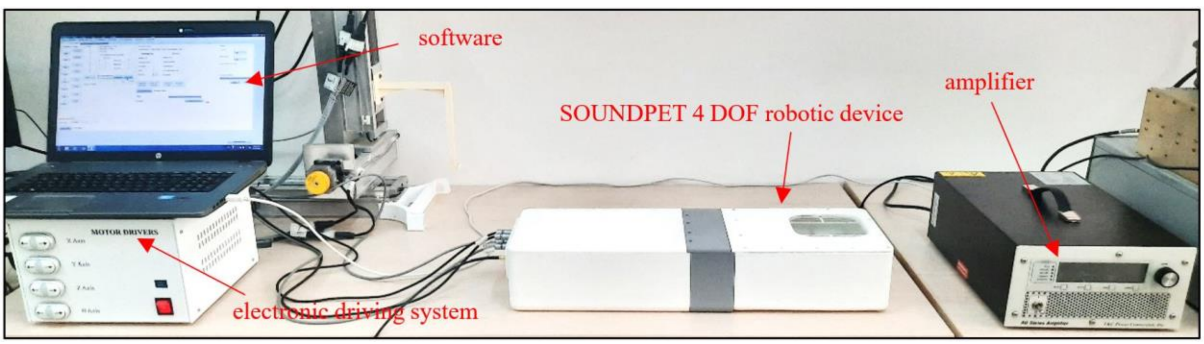

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

Figure 4: Animal positioning for the experiment.

Results

No lesions were observed from the side of the sonication (outer side) on the left thigh above and below the skin as shown in Figure 5 and Figure 6, respectively.

Figure 5: The outer side of the left thigh above the skin after the treatment.

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side).

Overlapping lesions were appeared on the inner side of the left thigh above and below the skin as shown in Figure 7 and Figure 8, respectively.

Figure 7: Macroscopic appearance of the overlapping lesions from the inner side of the left thigh and above the skin. The red arrow indicates the area of the created overlapping lesions on the skin.

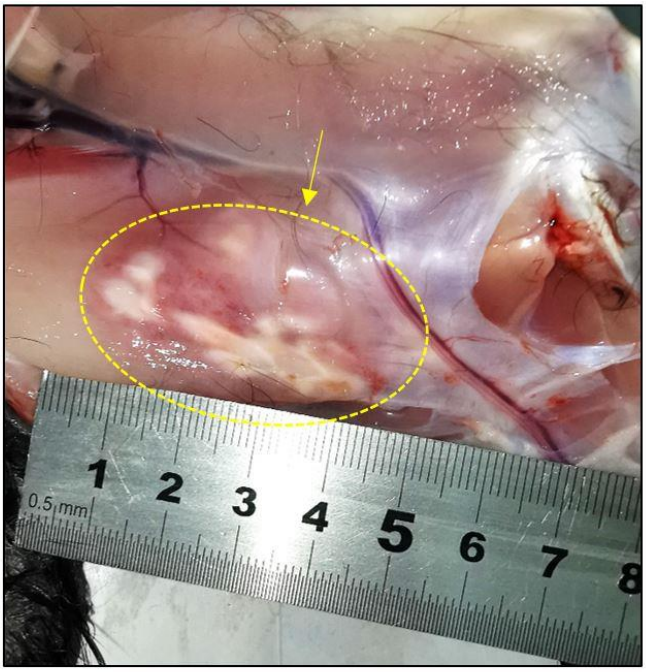

Figure 8: Macroscopic appearance of the exposed muscle from the inner side of the left thigh. Overlapping lesions were formed after performing a 4 x 4 grid at step movement of 5 mm. The yellow arrow indicates the area of the created overlapping lesions on the exposed muscle.

The muscle of the left thigh was dissected and a necrotic region was observed as shown in Figure 9.

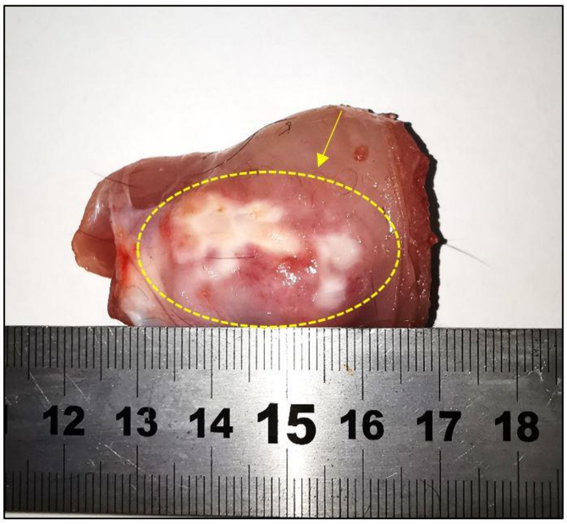

Figure 9: Macroscopic appearance of the overlapping lesions (indicated with yellow arrow) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 16 overlapping lesions on the surface of the muscle was calculated to be 25 mm x 13 mm while its depth was 13 mm. Figure 10 demonstrates the vertical cross middle dissection of the necrotic area that was created on the left thigh by applying a 4 x 4 grid pattern.

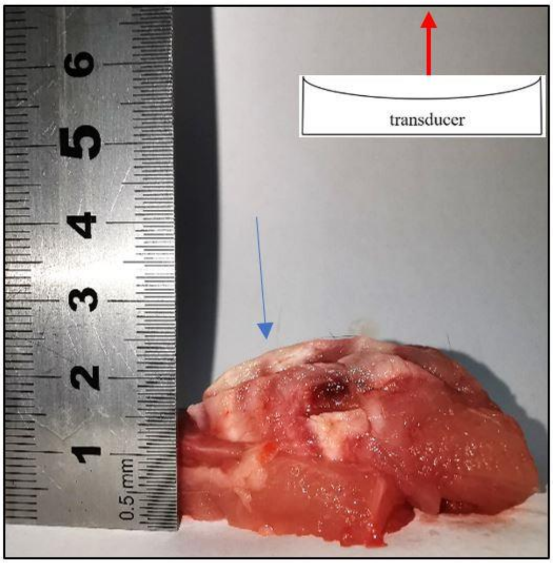

Figure 10: In situ dissection of the overlapping lesions that were created on the left thigh. The blue arrow indicates the necrotic area and the red arrow indicates the beam direction.

No lesions were observed from the side of the sonication (outer side) on the right thigh above and below the skin as shown in Figure 11 and Figure 12, respectively.

Figure 11: The outer side of the right thigh above the skin after the treatment.

Figure 12: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed.

Overlapping lesions were appeared on the inner side of the right thigh above and below the skin as shown in Figure 13 and Figure 14, respectively.

Figure 13: Macroscopic appearance of the overlapping lesions as observed from the inner side of the right thigh and above the skin. The red arrow indicates the area of the created overlapping lesions on the skin.

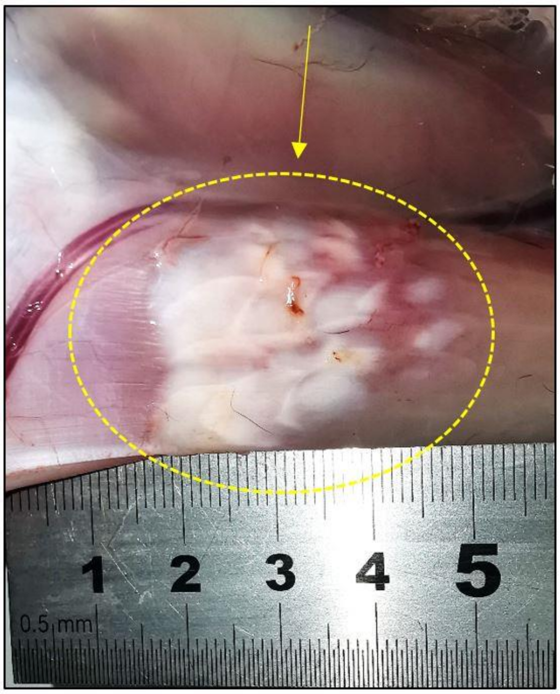

Figure 14: Macroscopic appearance of the overlapping lesions on the exposed muscle from the inner side of the right thigh. The yellow arrow indicates the formed lesions after performing a 4 x 4 grid at step movement of 5 mm.

The muscle of the right thigh was dissected and a necrotic region was observed as shown in Figure 15.

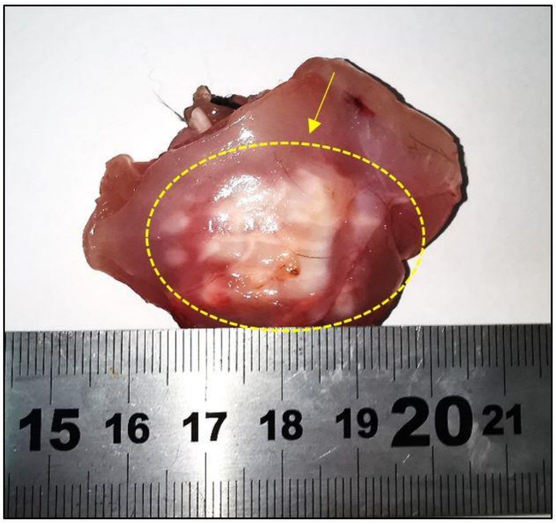

Figure 15: Macroscopic appearance of the formed lesions (indicated with yellow arrow) on the dissected muscle of the inner side of the right thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 16 overlapping lesions on the surface of the muscle was calculated to be 18 mm x 18 mm while its depth was 11 mm. Most of the formed lesions were overlapping but few of them around the necrotic region were discrete. Figure 16 demonstrates the vertical cross middle dissection of the overlapping lesions that were created on the right thigh by applying a 4 x 4 grid pattern and Figure 17 shows the dissection of the discrete lesions that were observed on the periphery of the main necrotic region. The average diameter and length of the discrete lesions was 3 mm and 4 mm, respectively.

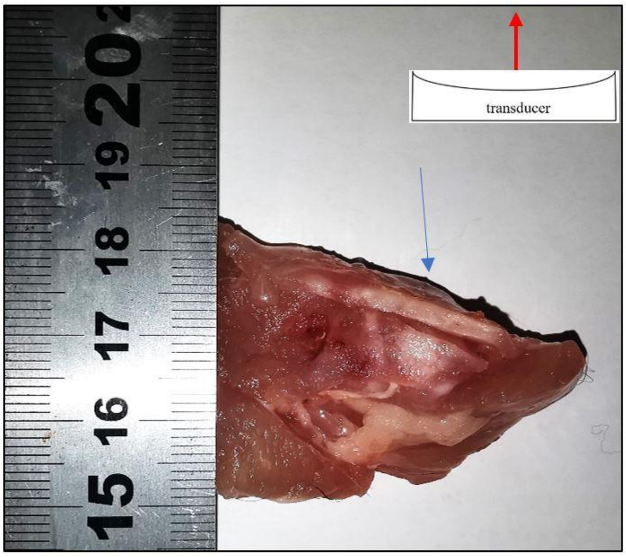

Figure 16: In situ dissection of the overlapping lesions on the right thigh. The blue arrow indicates the formed lesions and the red arrow indicates the beam direction.

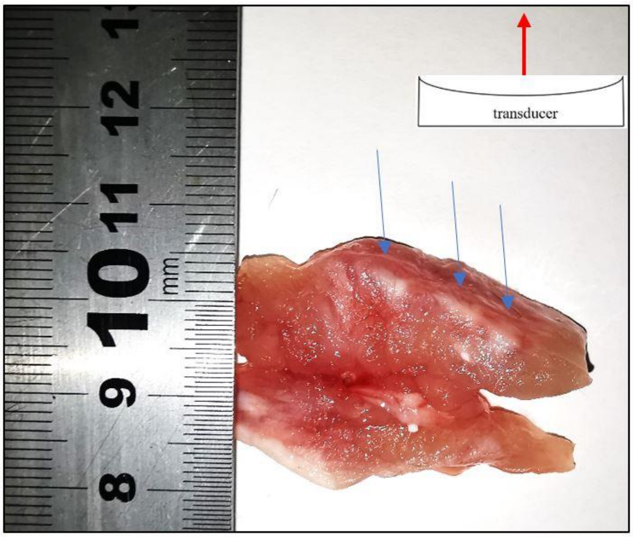

Figure 17: In situ dissection of the discrete lesions that were formed on the periphery of the main necrotic region on the right thigh. The blue arrows indicate the formed lesions and the red arrow indicates the beam direction.

Table 3 lists the anesthesia record for the rabbit experiment.

Table 3: Anesthesia record for rabbit 18.

Animal ID	SOUNDPET_2020_18				
Species	Rabbit				
Sex	M				
Age	Adult				
Weight (Kg)	2.1				
Supplier	S. Ioannou Farm (CYCU5.05)				
Grant	SOUNDPET (INTEGRATED/0918/0008)				
Study	CY/EXP/PR.L01/2020				
Participants	Kyriakos Spanoudes				
	Theocharis Drakos				
	Nikolas Evripidou				
Principal Investigator	Christakis Damianou				
Time	**Anesthesia**				
13:00	Medetomidine 1 ml + Ketamine 0.3 ml				
13:10	Medetomidine 0.5 ml				
Euthanasia	14:15 1 ml T-61				
Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
13:10	110	60	1	Absent	n/a
13:20	100	64	0	Absent	n/a
13:30	100	64	0	Absent	n/a
13:40	100	66	0	Absent	n/a
13:50	110	66	0	Absent	n/a
14:00	100	64	0	Absent	n/a
14:10	100	64	0	Absent	n/a

Rabbit 19

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	300
Acoustic power (W)	93
Sonication time (s)	5
Energy (J)	465
Cooling time (s)	90
Number of sonications	25
Step between sonications (mm)	5
Distance transducer/rabbit skin (cm)	7

A 5 x 5 grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 25 at step movement of 5 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

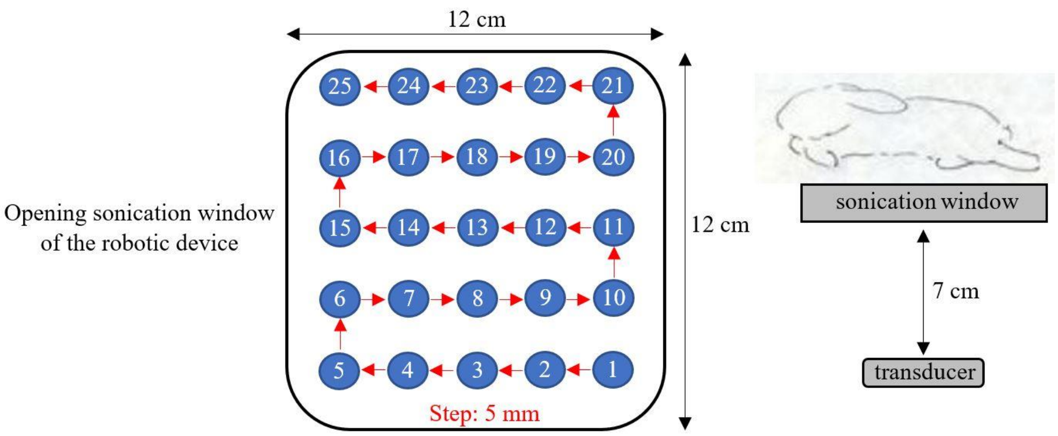

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	300
Acoustic power (W)	93
Sonication time (s)	3
Energy (J)	279
Cooling time (s)	90
Number of sonications	25
Step between sonications (mm)	4
Distance transducer/rabbit skin (cm)	7

A 5 x 5 grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved from point 1 to 25 at step movement of 4 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

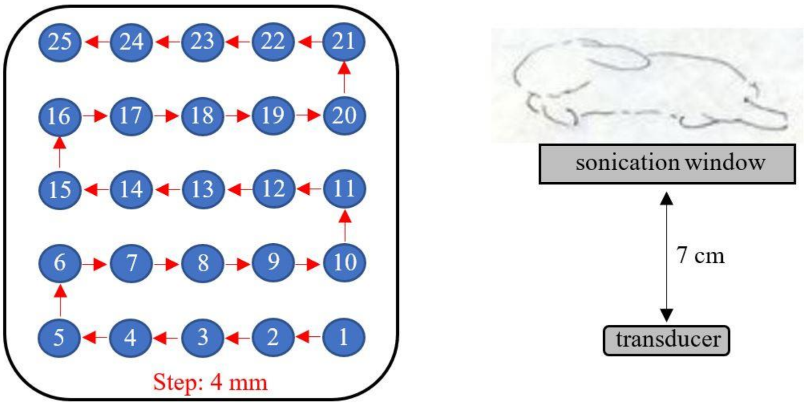

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory, that was used for the rabbit thigh ablations.

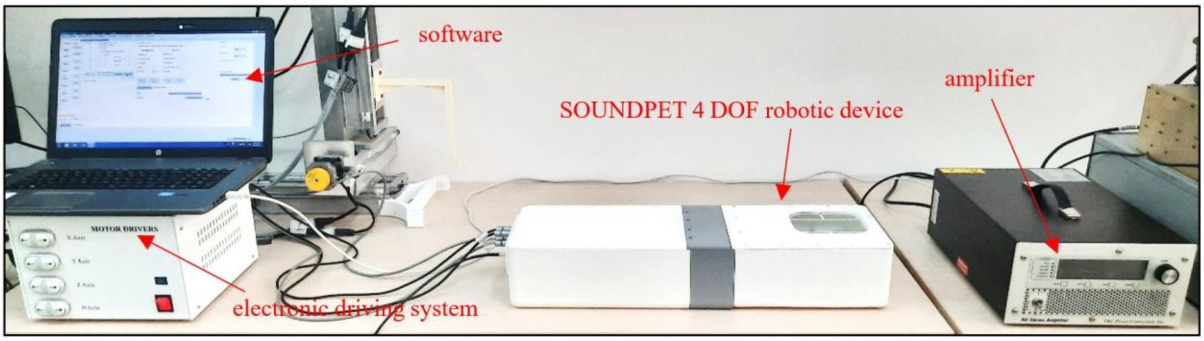

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

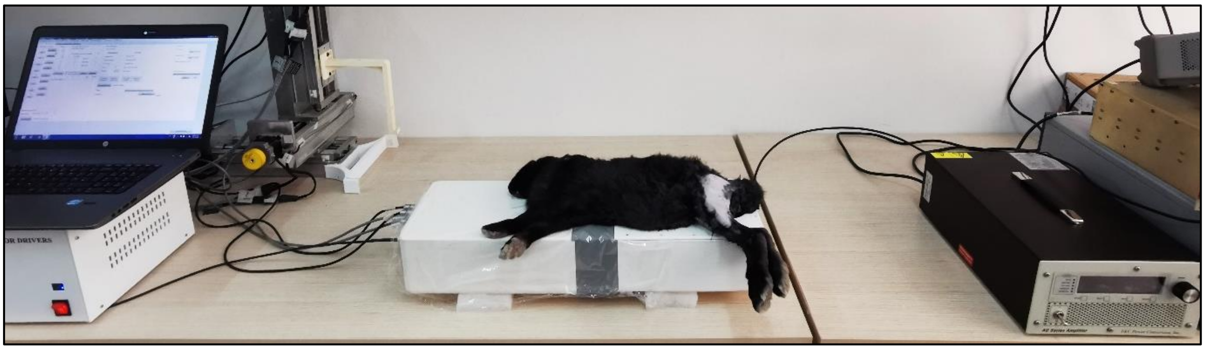

Figure 4: Animal positioning for the experiment.

Results

No lesions were observed from the side of the sonication (outer side) on the left thigh above and below the skin as shown in Figure 5 and Figure 6, respectively.

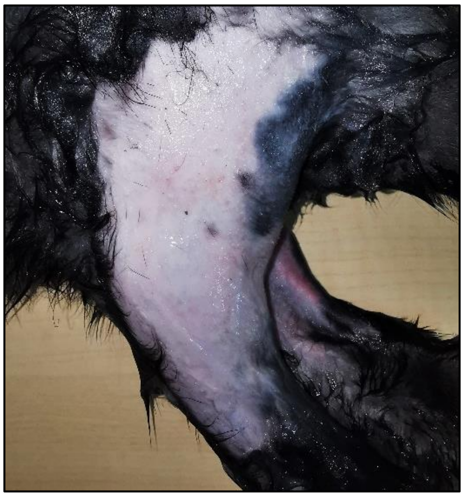

Figure 5: The outer side of the left thigh above the skin after the treatment.

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side).

Overlapping lesions were appeared on the inner side of the left thigh above and below the skin as shown in Figure 7 and Figure 8, respectively.

Figure 7: Macroscopic appearance of the overlapping lesions from the inner side of the left thigh and above the skin. The red arrow indicates the area of the created overlapping lesions on the skin.

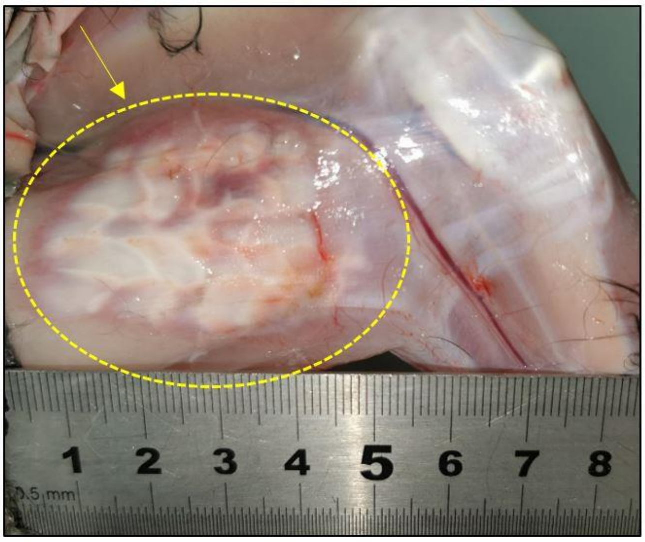

Figure 8: Macroscopic appearance of the exposed muscle from the inner side of the left thigh. Overlapping lesions were formed after performing a 5 x 5 grid at step movement of 5 mm. The yellow arrow indicates the area of the created overlapping lesions on the exposed muscle.

The muscle of the left thigh was dissected and a necrotic region was observed as shown in Figure 9.

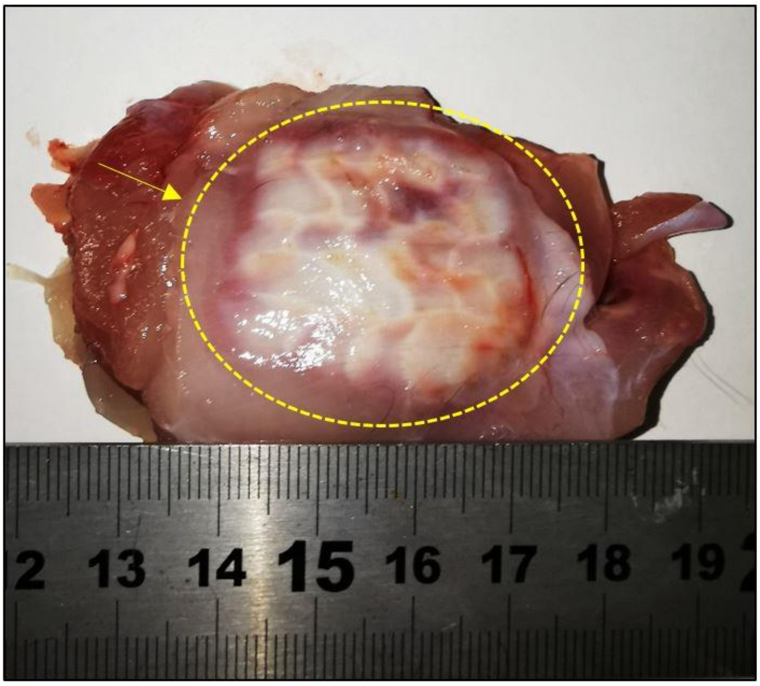

Figure 9: Macroscopic appearance of the overlapping lesions (indicated with yellow arrow) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 25 overlapping lesions on the surface of the muscle was calculated to be 30 mm x 26 mm while its depth was 10 mm. Figure 10 demonstrates the vertical cross middle dissection (with its mirror view) of the necrotic area that was created on the left thigh by applying a 5 x 5 grid pattern.

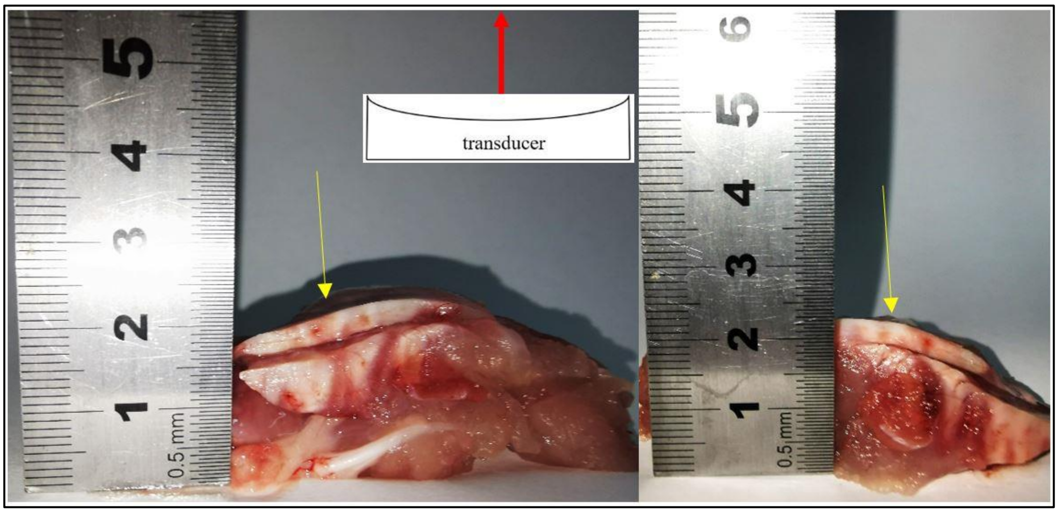

Figure 10: In situ dissection of the overlapping lesions that were created on the left thigh. The yellow arrows indicate the necrotic area and the red arrow indicates the beam direction.

No lesions were observed from the side of the sonication (outer side) on the right thigh above and below the skin as shown in Figure 11 and Figure 12, respectively.

Figure 11: The outer side of the right thigh above the skin after the treatment.

Figure 12: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed.

Overlapping lesions were appeared on the inner side of the right thigh above and below the skin as shown in Figure 13 and Figure 14, respectively.

Figure 13: Macroscopic appearance of the overlapping lesions as observed from the inner side of the right thigh and above the skin. The red arrow indicates the area of the created overlapping lesions on the skin.

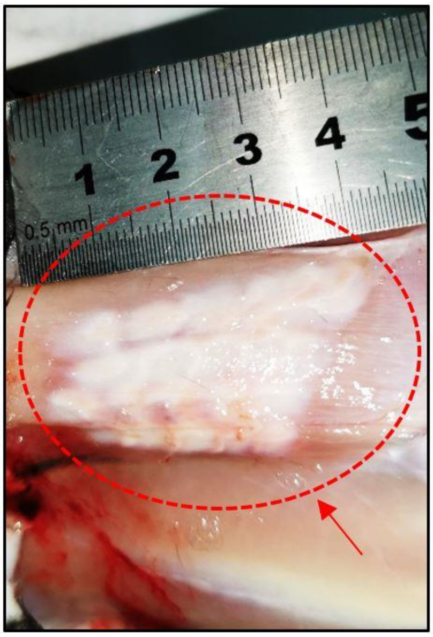

Figure 14: Macroscopic appearance of the overlapping lesions on the exposed muscle from the inner side of the right thigh. The red arrow indicates the formed lesions after performing a 5 x 5 grid at step movement of 4 mm.

The muscle of the right thigh was dissected and a necrotic region was observed as shown in Figure 15.

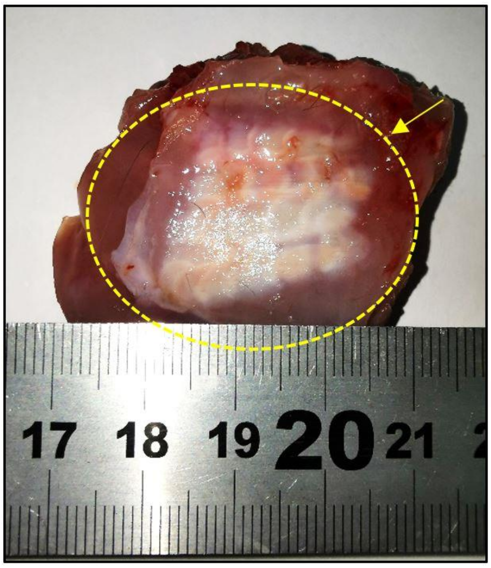

Figure 15: Macroscopic appearance of the formed lesions (indicated with yellow arrow) on the dissected muscle of the inner side of the right thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 25 overlapping lesions on the surface of the muscle was calculated to be 20 mm x 20 mm while its depth was 4 mm. Figure 16 demonstrates the vertical cross middle dissection (with its mirror view) of the necrotic area that was created on the right thigh by applying a 5 x 5 grid pattern.

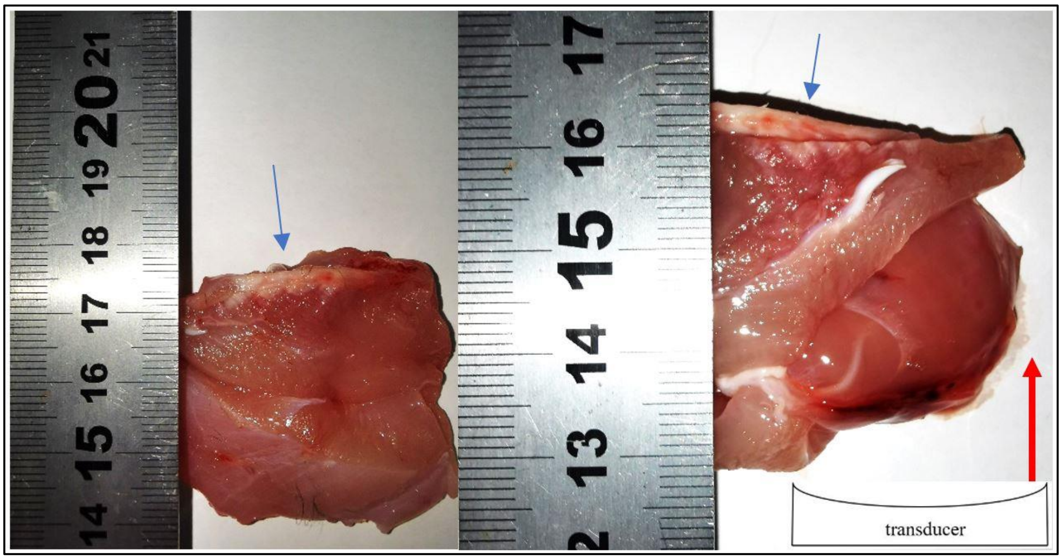

Figure 16: In situ dissection of the overlapping lesions on the right thigh. The blue arrows indicate the formed lesions and the red arrow indicates the beam direction.

Table 3 lists the anesthesia record for the rabbit experiment.

Table 3: Anesthesia record for rabbit 19.

Animal ID	SOUNDPET_2020_19				
Species	Rabbit				
Sex	F				
Age	Adult				
Weight (Kg)	2.25				
Supplier	S. Ioannou Farm (CYCU5.05)				
Grant	SOUNDPET (INTEGRATED/0918/0008)				
Study	CY/EXP/PR.L01/2020				
Participants	Kyriakos Spanoudes				
	Theocharis Drakos				
	Nikolas Evripidou				
Principal Investigator	Christakis Damianou				
Time	**Anesthesia**				
09:35	Medetomidine 1 ml + Ketamine 0.3 ml				
09:50	Medetomidine 0.4 ml				
Euthanasia	11:05 1 ml T-61				
Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
09:40	120	62	0	Absent	n/a
09:50	110	66	0	Absent	n/a
10:10	120	62	0	Absent	n/a

10:30	110	62	0	Absent	n/a
10:40	110	64	0	Absent	n/a
11:00	110	64	0	Absent	n/a

Rabbit 20

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	350
Acoustic power (W)	108.5
Sonication time (s)	5
Energy (J)	542.5
Cooling time (s)	90
Number of sonications	25
Step between sonications (mm)	5
Distance transducer/rabbit skin (cm)	7

A 5 x 5 grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 25 at step movement of 5 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

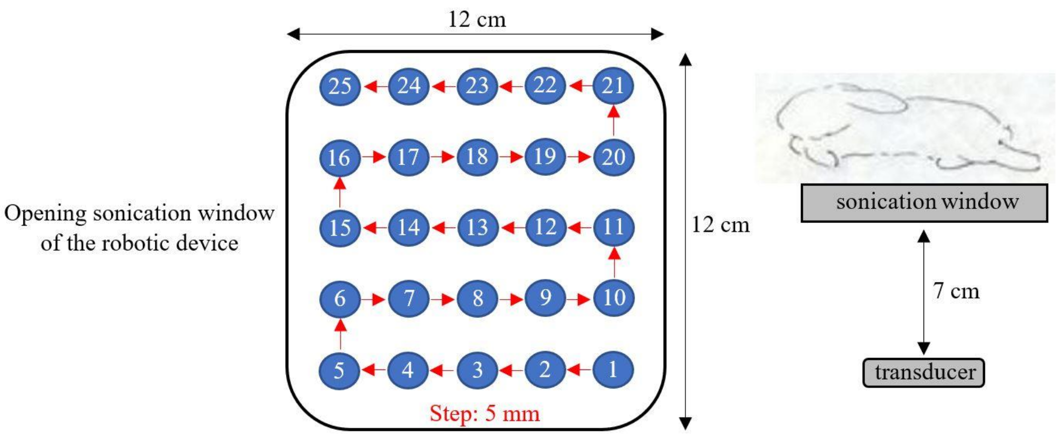

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	350
Acoustic power (W)	108.5
Sonication time (s)	3
Energy (J)	325.5
Cooling time (s)	90
Number of sonications	25
Step between sonications (mm)	4
Distance transducer/rabbit skin (cm)	7

A 5 x 5 grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved from point 1 to 25 at step movement of 4 mm and the distance between the transducer and the opening sonicated window of the robotic device was 7 cm.

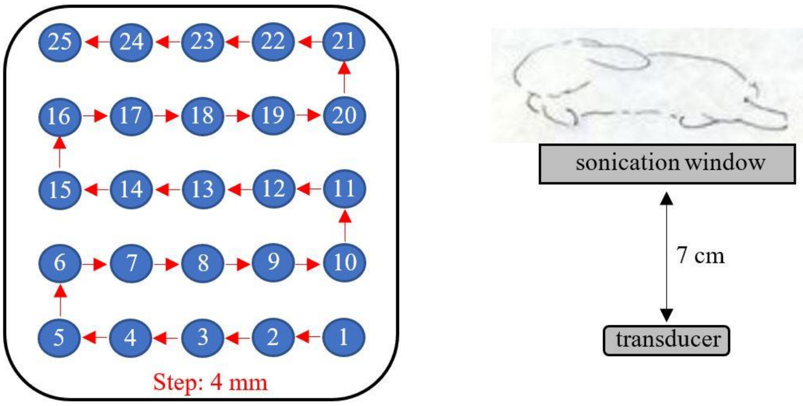

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory, that was used for the rabbit thigh ablations.

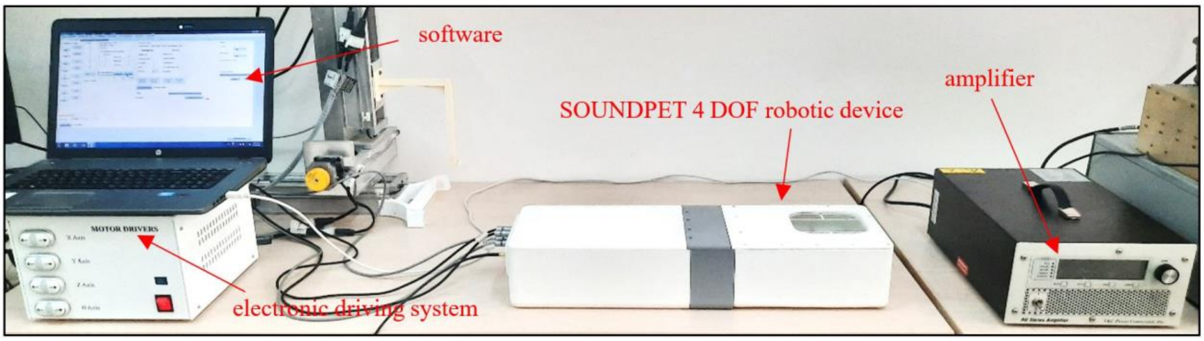

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

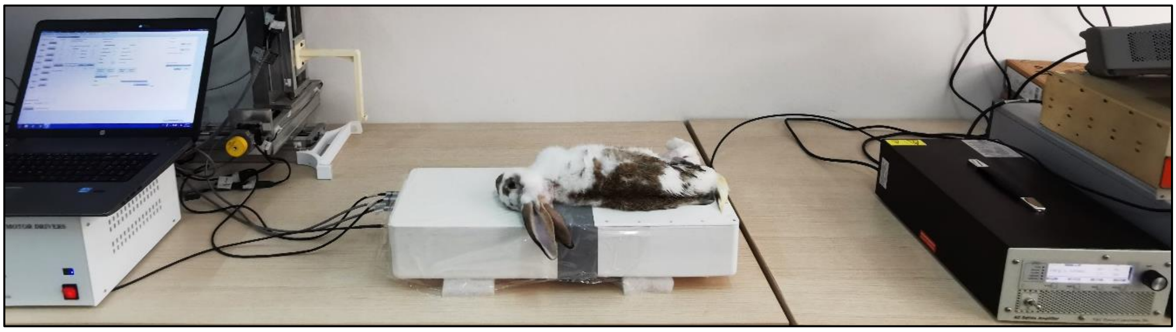

Figure 4: Animal positioning for the experiment.

Results

No lesions were observed from the side of the sonication (outer side) on the left thigh above and below the skin as shown in Figure 5 and Figure 6, respectively.

Figure 5: The outer side of the left thigh above the skin after the treatment.

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side).

Overlapping lesions were appeared on the inner side of the left thigh above and below the skin as shown in Figure 7 and Figure 8, respectively.

Figure 7: Macroscopic appearance of the overlapping lesions from the inner side of the left thigh and above the skin. The red arrow indicates the area of the created overlapping lesions on the skin.

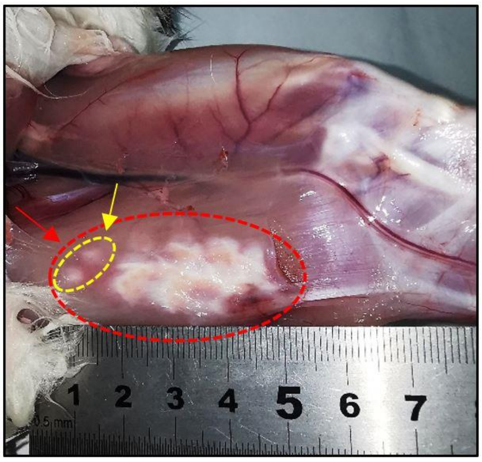

Figure 8: Macroscopic appearance of the exposed muscle from the inner side of the left thigh. Overlapping lesions were formed after performing a 5 x 5 grid at step movement of 5 mm. The red arrow indicates the area of the created overlapping lesions on the exposed muscle. Some lesions on the periphery of the necrotic region were discrete (indicated with yellow arrow).

The muscle of the left thigh was dissected and a necrotic region was observed as shown in Figure 9.

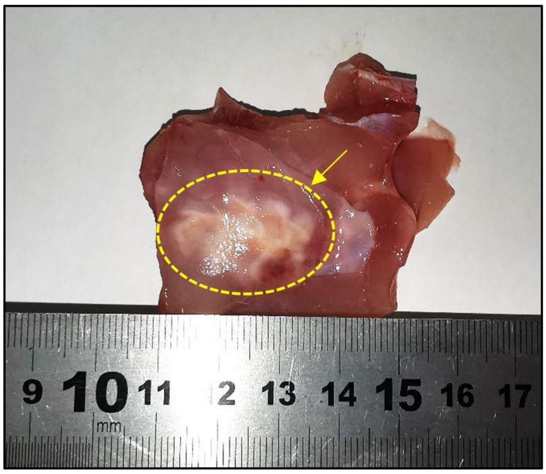

Figure 9: Macroscopic appearance of the overlapping lesions (indicated with yellow arrow) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 25 overlapping lesions on the surface of the muscle was calculated to be 23 mm x 15 mm while its depth was 17 mm. Figure 10 demonstrates the vertical cross middle dissection of the necrotic area that was created on the left thigh by applying a 5 x 5 grid pattern.

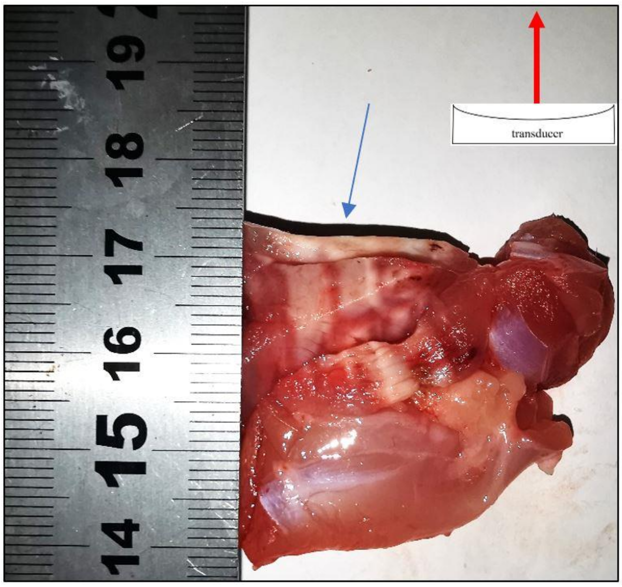

Figure 10: In situ dissection of the overlapping lesions that were created on the left thigh. The blue arrow indicates the necrotic area and the red arrow indicates the beam direction.

No lesions were observed from the side of the sonication (outer side) on the right thigh above and below the skin as shown in Figure 11 and Figure 12, respectively.

Figure 11: The outer side of the right thigh above the skin after the treatment.

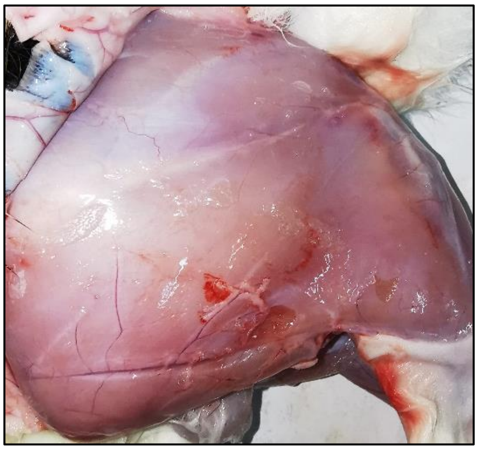

Figure 12: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed.

Overlapping lesions were appeared on the inner side of the right thigh above and below the skin as shown in Figure 13 and Figure 14, respectively.

Figure 13: Macroscopic appearance of the overlapping lesions as observed from the inner side of the right thigh and above the skin. The red arrow indicates the area of the created overlapping lesions on the skin.

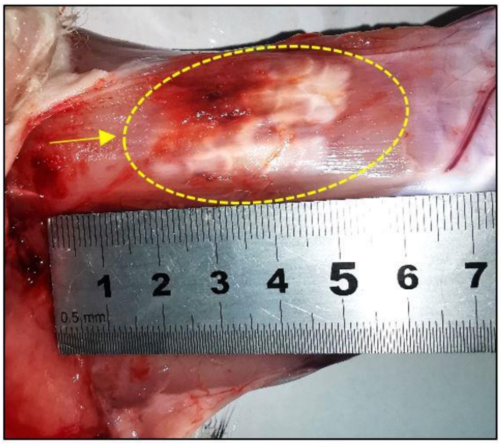

Figure 14: Macroscopic appearance of the overlapping lesions on the exposed muscle from the inner side of the right thigh. The yellow arrow indicates the formed lesions after performing a 5 x 5 grid at step movement of 4 mm.

The muscle of the right thigh was dissected and a necrotic region was observed as shown in Figure 15.

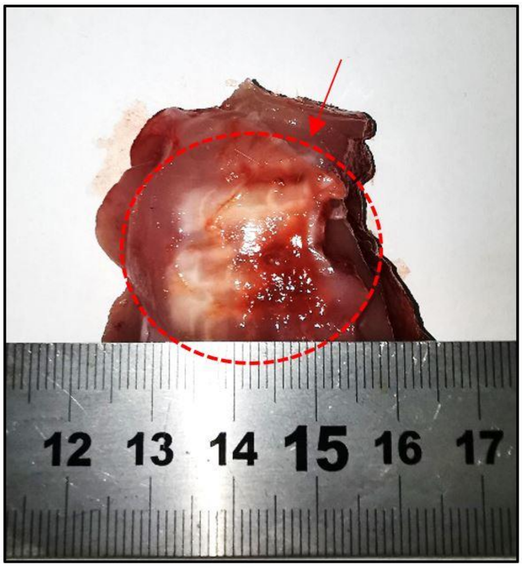

Figure 15: Macroscopic appearance of the formed lesions (indicated with red arrow) on the dissected muscle of the inner side of the right thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 25 overlapping lesions on the surface of the muscle was calculated to be 17 mm x 17 mm while its depth was 5 mm. Figure 16 demonstrates the vertical cross middle dissection (with its mirror view) of the necrotic area that was created on the right thigh by applying a 5 x 5 grid pattern.

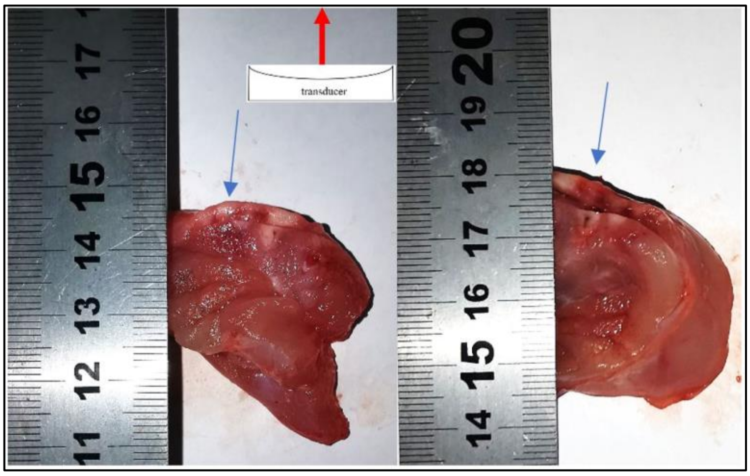

Figure 16: In situ dissection of the overlapping lesions on the right thigh. The blue arrows indicate the formed lesions and the red arrow indicates the beam direction.

Table 3 lists the anesthesia record for the rabbit experiment.

Table 3: Anesthesia record for rabbit 20.

Animal ID	SOUNDPET_2020_20
Species	Rabbit
Sex	M
Age	Adult
Weight (Kg)	2.3
Supplier	S. Ioannou Farm (CYCU5.05)
Grant	SOUNDPET (INTEGRATED/0918/0008)
Study	CY/EXP/PR.L01/2020
Participants	Kyriakos Spanoudes
	Theocharis Drakos
	Nikolas Evripidou
Principal Investigator	Christakis Damianou
Time	**Anesthesia**
11:45	Medetomidine 1.2 ml + Ketamine 0.4 ml
11:50	Midazolam
Euthanasia	12:50 1 ml T-61

Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
11:50	110	62	2	Absent	n/a
12:05	110	58	0	Absent	n/a
12:20	100	56	0	Absent	n/a
12:30	100	56	0	Absent	n/a
12:40	110	58	0	Absent	n/a
12:50	108	62	0	Absent	n/a

Appendix 2 – SOUNDPET robotic system 2nd version – Rabbit *in vivo* experiments (21-40)

1. Purpose

To assess the ability of the SOUNDPET robotic device (2nd version) to ablate the thigh of a living rabbit, find the minimum ultrasonic energy that is needed to create thermal lesions in rabbit tissue and test the movement accuracy of the robotic device.

2. Experimental materials and procedure

2.1 Experimental materials

The following experimental materials were used:

- Amplifier: AG1016 (AG Series Amplifier, T & C Power Conversion, Inc., Rochester, US)
- Tissue: Thigh (Live Rabbit)
- Transducer: ID 24 (frequency: 2.6 MHz, diameter: 38 mm, radius of curvature: 61 mm, efficiency: 23 %)
- SOUNDPET 4 DOF robotic system (2nd version)
- Electronic driving system (for large motors)
- MRgFUS software.

2.2 *In vivo* experiment

All experiments were approved by the authorities of the Veterinary Services, Ministry of Agriculture (CY/EXP/PR.L01/2020). Locally bred rabbits (purchased from an accredited farm; S. Ioannou-CYCU5.05) were examined, weighed, and anesthetized (see anesthesia record in each individual report). The thighs of each rabbit were depilated (VEET, Reckitt Benkiser, UK) shaved and each time the rabbit was placed on the robotic device with its thigh immersed in the degassed water above the transducer. The sonications were performed on the outer side of the thighs (left and right) of each rabbit.

3. Individual reports

Rabbit 21

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou, C. Damianou

Settings for thigh ablations

Sonications were performed on the left thigh of the rabbit for optimization of the transducer settings and the robotic device.

Table 1 lists the settings that were used for the sonications on the right thigh.

Table 1: Settings for right thigh.

Parameter	Value
Electric power (W)	200
Acoustic power (W)	46
Sonication time (s)	30
Energy (J)	1380
Cooling time (s)	60
Number of sonications	1
Distance transducer/rabbit skin (cm)	5

A single sonication was chosen for the right thigh ablation. The purpose was to check the transducer ability to create lesion and observe its dimensions for the selected ultrasonic parameters. The distance between the transducer and the opening sonicated window of the robotic device was 5 cm.

Experimental set-up

Figure 1 shows the experimental set-up in the laboratory that was used for the rabbit thigh ablations.

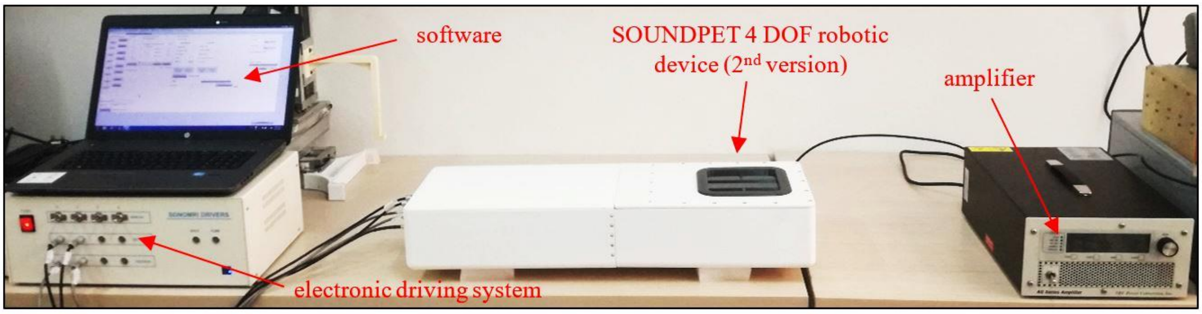

Figure 1: Experimental set-up for rabbit thigh ablation.

Results

Sonications were performed on the left thigh of the rabbit for optimization of the transducer settings and the robotic device. Therefore, no lesions were created on the left thigh.

A lesion was observed from the side of the sonication (outer side) on the right thigh above and below the skin as shown in Figure 2 and Figure 3, respectively.

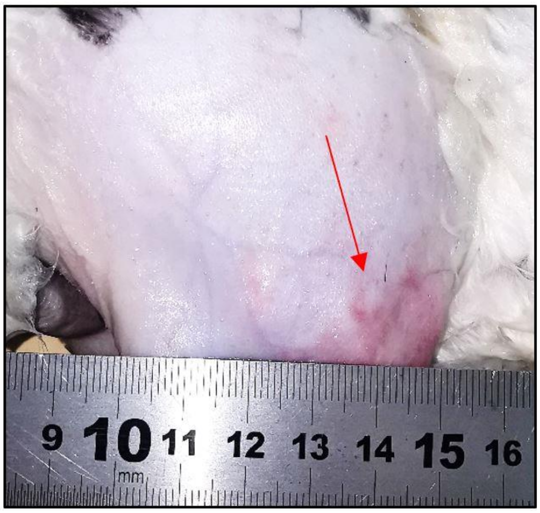

Figure 2: The outer side of the right thigh above the skin after the treatment. The red arrow indicates the formed lesion.

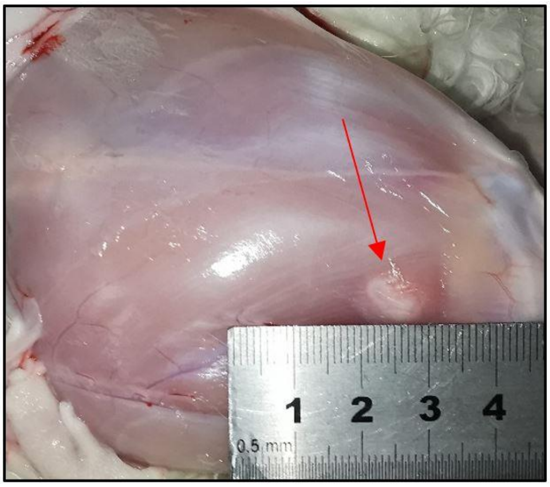

Figure 3: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed. The red arrow indicates the formed lesion.

The lesion was visible on the inner side of the right thigh above and below the skin as shown in Figure 4 and Figure 5.

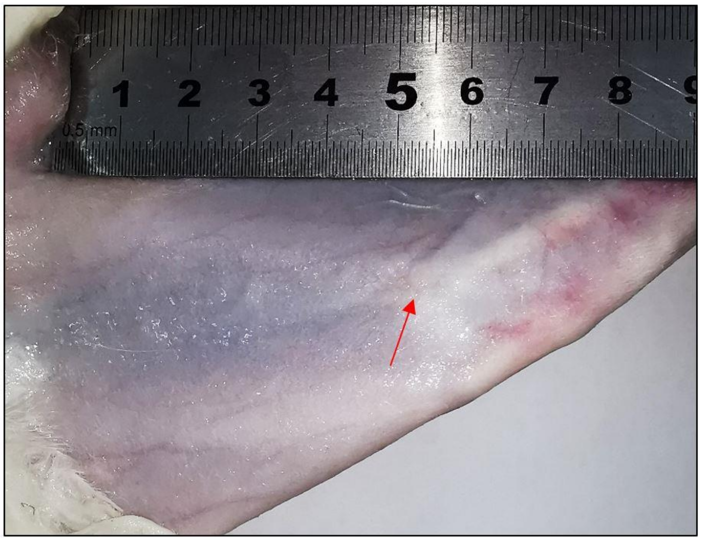

Figure 4: Macroscopic appearance of the lesion as observed from the inner side of the right thigh and above the skin. The red arrow indicates the lesion that was observed on the skin.

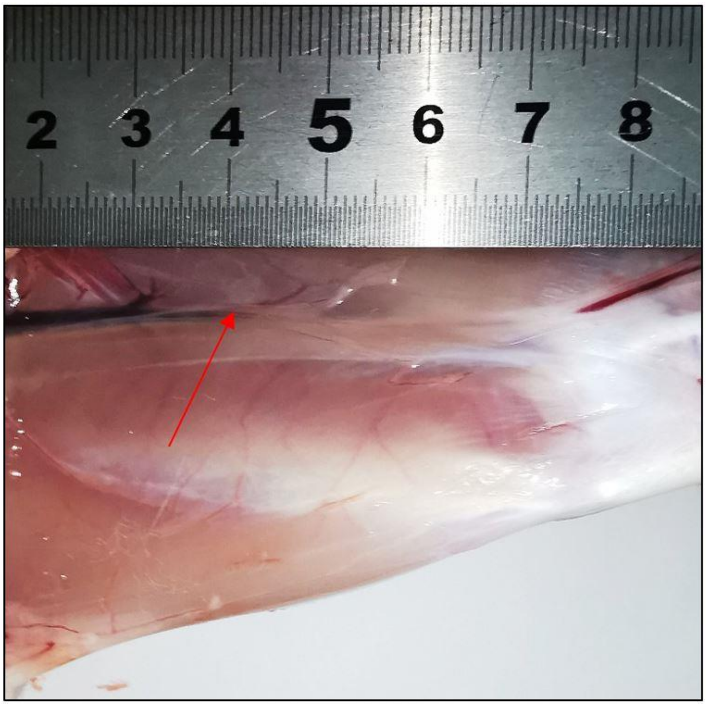

Figure 5: Macroscopic appearance of the lesion on the exposed muscle from the inner side of the right thigh. The red arrow indicates the formed lesion.

The muscle of the right thigh was dissected and the lesion was observed as shown in Figure 6.

Figure 6: Macroscopic appearance of the formed lesion (indicated with yellow arrow) on the dissected muscle of the inner side of the right thigh (plane perpendicular to the beam).

Figure 7 demonstrates the vertical cross middle dissection of the lesion that was created on the right thigh.

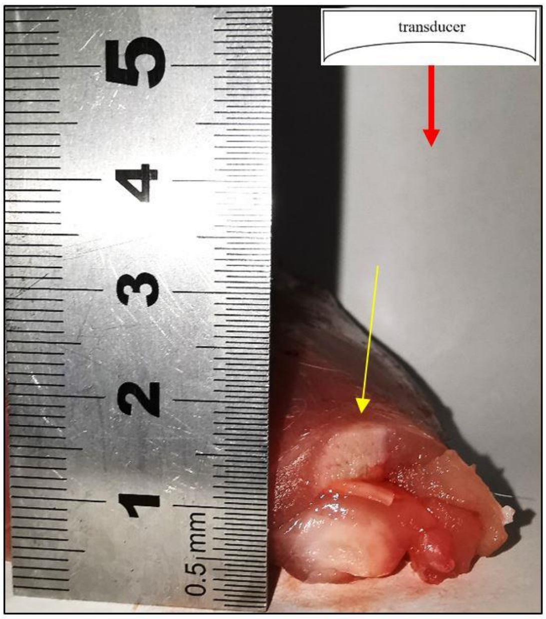

Figure 7: In situ dissection of the formed lesion of the right thigh. The yellow arrow indicates the formed lesion and the red arrow indicates the beam direction.

The diameter and length of the lesion was 5 mm and 7 mm, respectively. Table 2 lists the anesthesia record for the rabbit experiment.

Table 2: Anesthesia record for rabbit 21.

Animal ID	SOUNDPET_2020_21				
Species	Rabbit				
Sex	M				
Age	Adult				
Weight (Kg)	2.1				
Supplier	S. Ioannou Farm (CYCU5.05)				
Grant	SOUNDPET (INTEGRATED/0918/0008)				
Study	CY/EXP/PR.L01/2020				
Participants	Kyriakos Spanoudes				
	Theocharis Drakos				
	Nikolas Evripidou				
	Christakis Damianou				
Principal Investigator	Christakis Damianou				
Time	**Anesthesia**				
12:00	Medetomidine 1 ml + Ketamine 0.3 ml				
12:10	Midazolam 0.5 ml				
Euthanasia	13:00 1 ml T-61				
Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
12:00	100	60	0	Absent	n/a
12:10	90	72	1	Absent	n/a
12:20	90	68	0	Absent	n/a
12:30	100	68	0	Absent	n/a
12:45	110	64	0	Absent	n/a
12:55	100	68	0	Absent	n/a

Rabbit 22

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou, C. Damianou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	100
Acoustic power (W)	23
Sonication time (s)	10, 20, 30
Energy (J)	230, 460, 690
Cooling time (s)	60
Number of sonications	3
Step between sonications (mm)	15
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 3 (direction from rabbit feet to tail) at step movement of 15 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm.

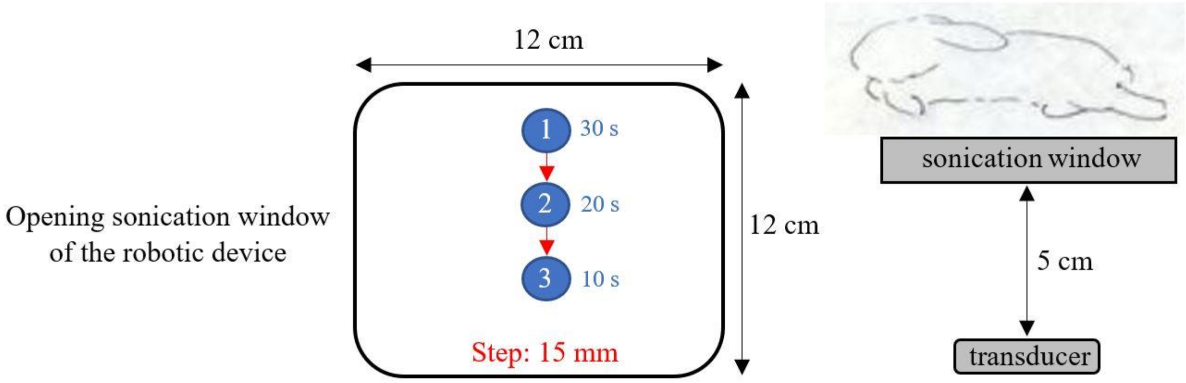

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	100
Acoustic power (W)	23
Sonication time (s)	10, 20, 30
Energy (J)	230, 460, 690
Cooling time (s)	60
Number of sonications	3
Step between sonications (mm)	15
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved from point 1 to 3 (direction from rabbit feet to tail) at step movement of 15 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm.

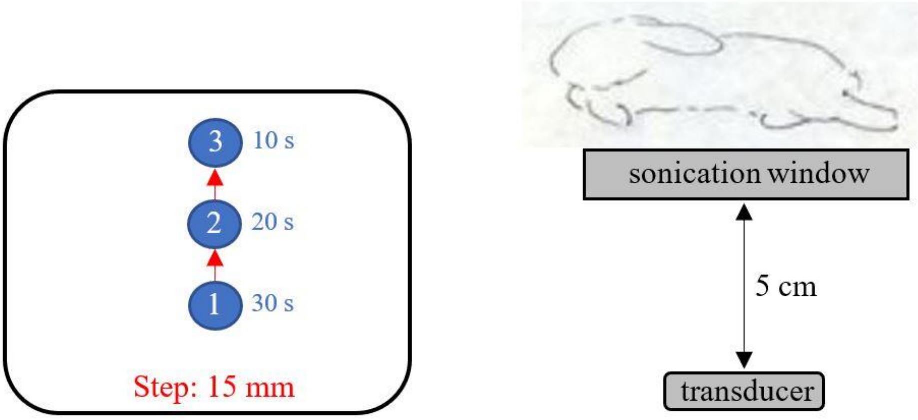

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory, that was used for the rabbit thigh ablations.

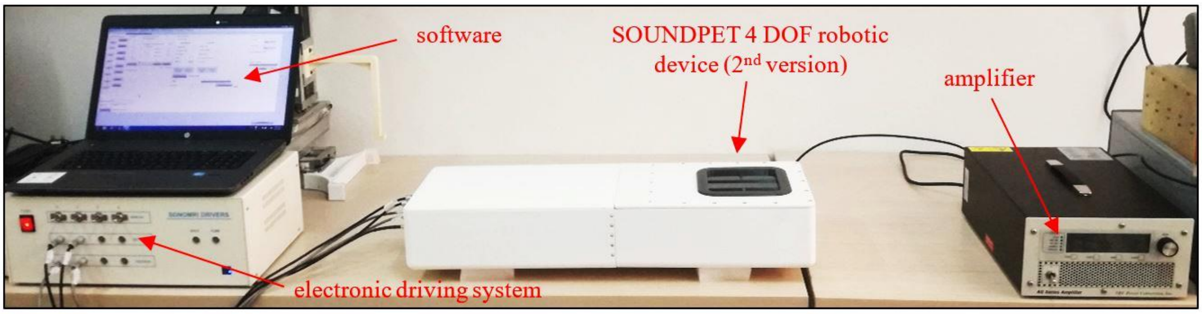

Figure 3: Experimental set-up for rabbit thigh ablation.

Results

As shown in Figure 4, no lesions were appeared on the outer side (sonication side) of the left thigh above the skin while a single lesion was observed below the skin as shown in Figure 5.

Figure 4: The outer side of the left thigh above the skin after the treatment.

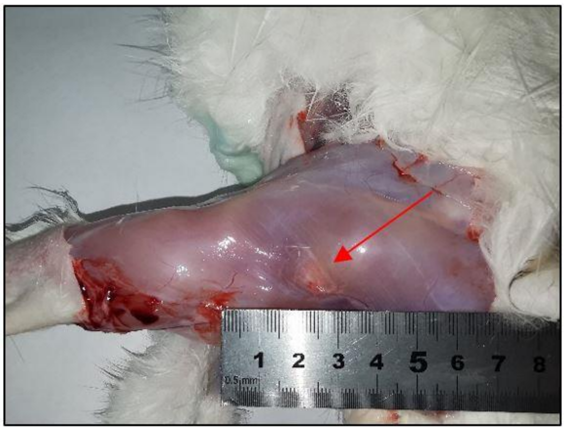

Figure 5: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side). The red arrow indicates the formed lesion.

Discrete lesions were observed on the inner side of the left thigh both above and below the skin as shown in Figure 6 and Figure 7, respectively.

Figure 6: Macroscopic appearance of the discrete lesions from the inner side of the left thigh and above the skin. The red arrows indicate the formed lesions on the skin.

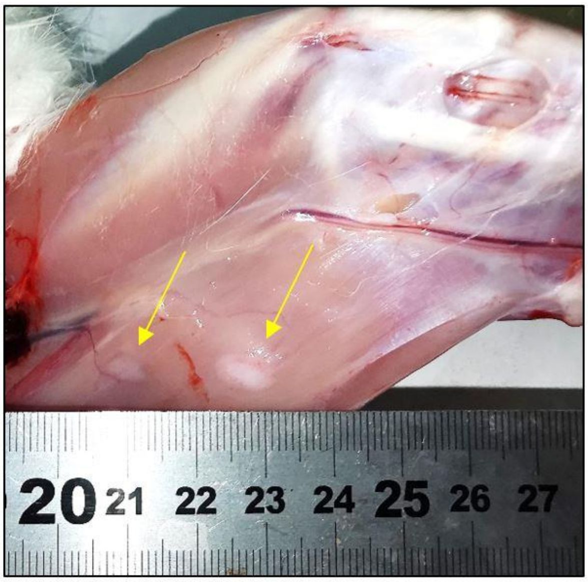

Figure 7: Macroscopic appearance of the discrete lesions on the exposed muscle from the inner side of the left thigh. The yellow arrows indicate the lesions after performing a linear grid of 3 sonications of different acoustic energy at step movement of 15 mm.

The muscle of the left thigh was dissected and two faintly discrete lesions were observed as shown in Figure 8.

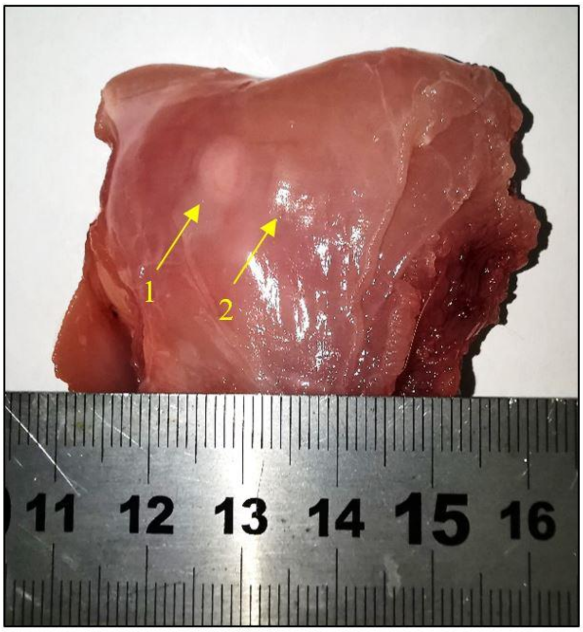

Figure 8: Macroscopic appearance of the formed lesions (indicated with yellow arrows) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The diameter of the formed lesions (two lesions were visible after the muscle dissection) was measured to be 6 mm and 4 mm for lesion 1 and 2, respectively. The depth of the lesion 1 and 2 was 11 mm and 9 mm, respectively. Figure 9 and Figure 10 demonstrates the dissection of the lesion 1 and 2 that were created on the left thigh, respectively. Lesion 1 was created by a

30 s sonication while the lesion 2 was created by a 20 s sonication. The 10 s sonication and the total energy was not enough to create a lesion.

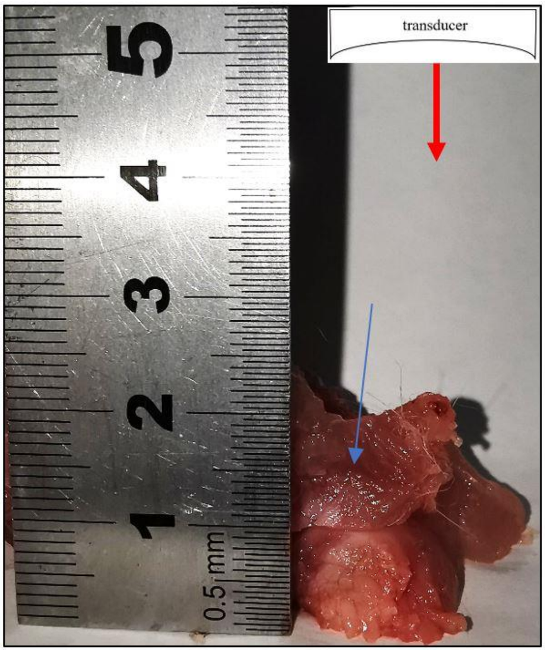

Figure 9: In situ dissection of the formed lesion 1 (30 s sonication) of the left thigh. The blue arrow indicates the formed lesion and the red arrow indicates the beam direction.

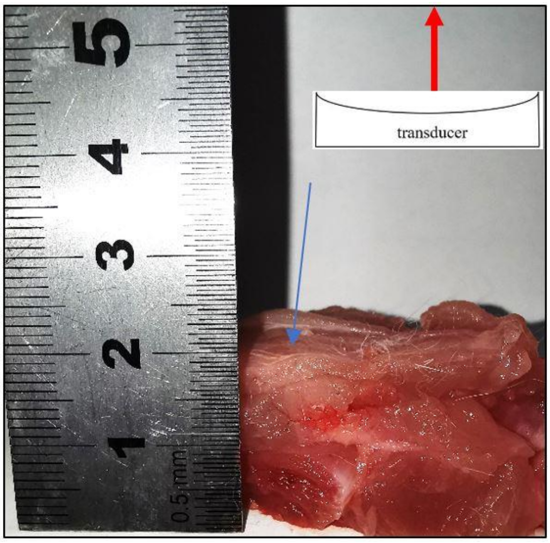

Figure 10: In situ dissection of the formed lesion 2 (20 s sonication) of the left thigh. The blue arrow indicates the formed lesion and the red arrow indicates the beam direction.

Two (2) lesions were observed on the left thigh after the treatment (Table 3). The diameter and length of the two lesions was measurable.

Table 3: Summary of results for the left thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	6	11
2	4	9
3	-	-

No lesions were observed from the side of the sonication (outer side) on the right thigh above the skin and one lesion was observed below the skin as shown in Figure 11 and Figure 12, respectively.

Figure 11: The outer side of the right thigh above the skin after the treatment.

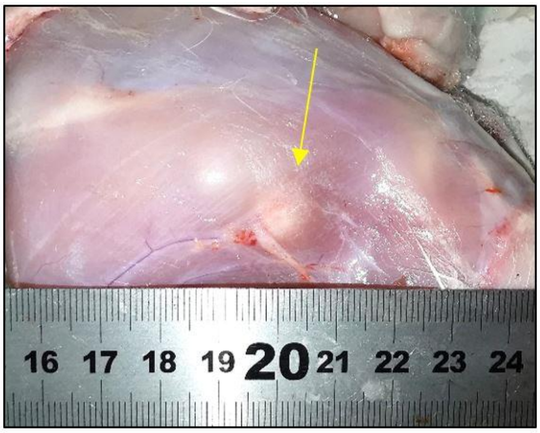

Figure 12: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed. The yellow arrow indicates the formed lesion.

The lesion was also appeared on the inner side of the right thigh above and below the skin as shown in Figure 13 and Figure 14, respectively.

Figure 13: Macroscopic appearance of the lesion as observed from the inner side of the right thigh and above the skin. The red arrow indicate the lesion that was observed on the skin.

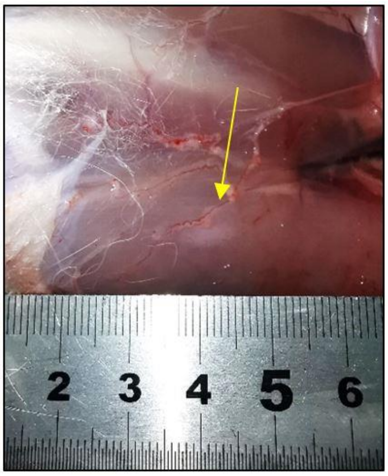

Figure 14: Macroscopic appearance of the lesion on the exposed muscle from the inner side of the right thigh. The yellow arrow indicates the formed lesion that was possibly created by a sonication of 30 s.

The muscle of the right thigh was dissected and two discrete lesions were observed as shown in Figure 15.

Figure 15: Macroscopic appearance of the formed lesions (indicated with red arrows) on the dissected muscle of the inner side of the right thigh (plane perpendicular to the beam).

Figure 16 demonstrates the vertical cross middle dissection of the lesions that were created on the right thigh.

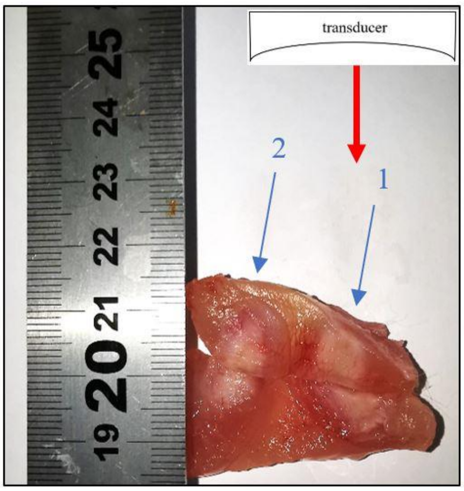

Figure 16: In situ dissection of the formed lesions of the right thigh. The blue arrows indicate the formed lesions and the red arrow indicates the beam direction.

Two (2) lesions were observed on the right thigh after the treatment (Table 4). The diameter and length of the two lesions was measurable.

Table 4: Summary of results for the right thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	9	16
2	7	14
3	-	-

Table 5 lists the anesthesia record for the rabbit experiment.

Table 5: Anesthesia record for rabbit 22.

Animal ID	SOUNDPET_2020_22				
Species	Rabbit				
Sex	M				
Age	Adult				
Weight (Kg)	2.0				
Supplier	S. Ioannou Farm (CYCU5.05)				
Grant	SOUNDPET (INTEGRATED/0918/0008)				
Study	CY/EXP/PR.L01/2020				
Participants	Kyriakos Spanoudes				
	Theocharis Drakos				
	Nikolas Evripidou				
	Christakis Damianou				
Principal Investigator	Christakis Damianou				
Time	**Anesthesia**				
12:30	Medetomidine 1 ml + Ketamine 0.3 ml				
12:40	Midazolam 0.5 ml				
Euthanasia	13:10 1 ml T-61				
Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
12:30	110	72	0	Absent	n/a
12:40	116	72	0	Absent	n/a
12:50	116	68	0	Absent	n/a
13:00	112	72	0	Absent	n/a

Rabbit 23

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	150
Acoustic power (W)	34.5
Sonication time (s)	10, 20, 30
Energy (J)	345, 690, 1035
Cooling time (s)	60
Number of sonications	3
Step between sonications (mm)	15
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 3 (direction from rabbit feet to tail) at step movement of 15 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm.

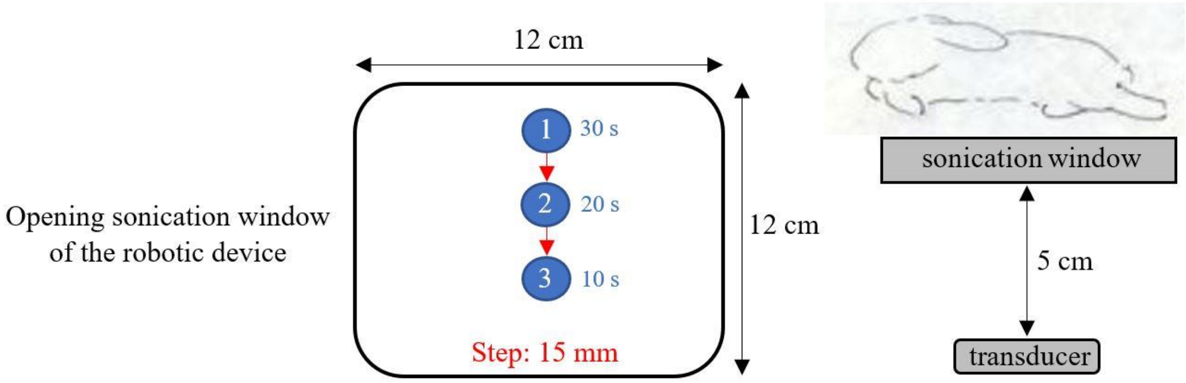

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	150
Acoustic power (W)	34.5
Sonication time (s)	10, 20, 30
Energy (J)	345, 690, 1035
Cooling time (s)	60
Number of sonications	3
Step between sonications (mm)	15
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved from point 1 to 3 (direction from rabbit feet to tail) at step movement of 15 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm.

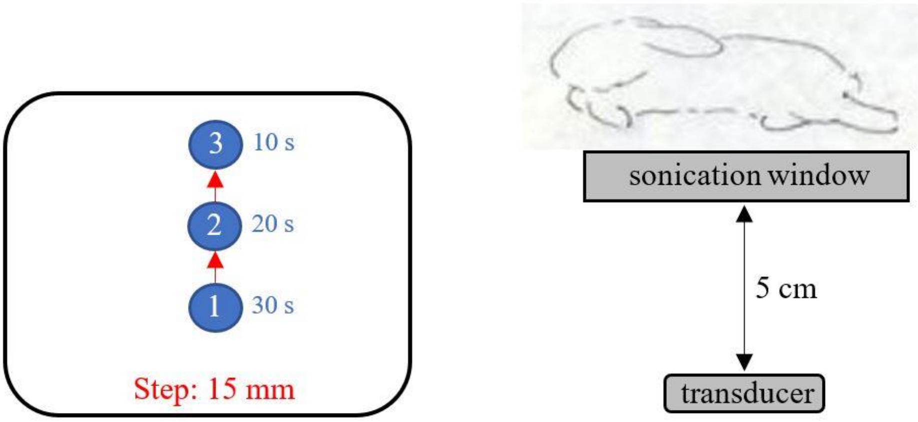

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory, that was used for the rabbit thigh ablations.

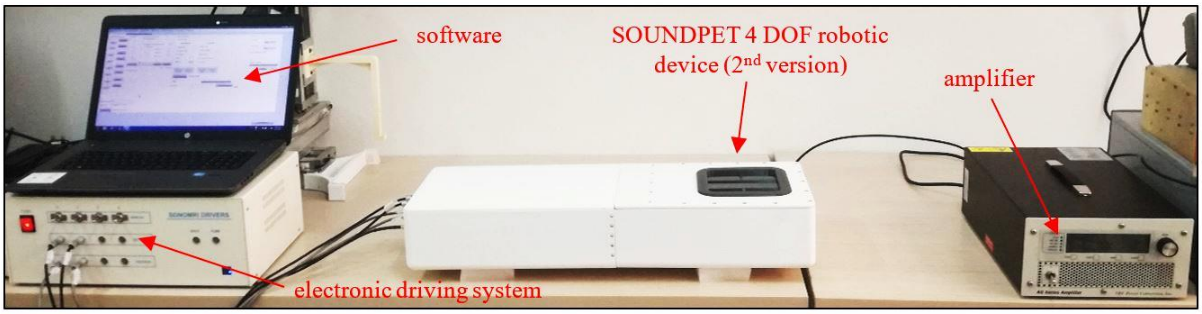

Figure 3: Experimental set-up for rabbit thigh ablation.

Results

As shown in Figure 4, no lesions were appeared on the outer side (sonication side) of the left thigh above the skin while two discrete lesions were observed below the skin as shown in Figure 5.

Figure 4: The outer side of the left thigh above the skin after the treatment.

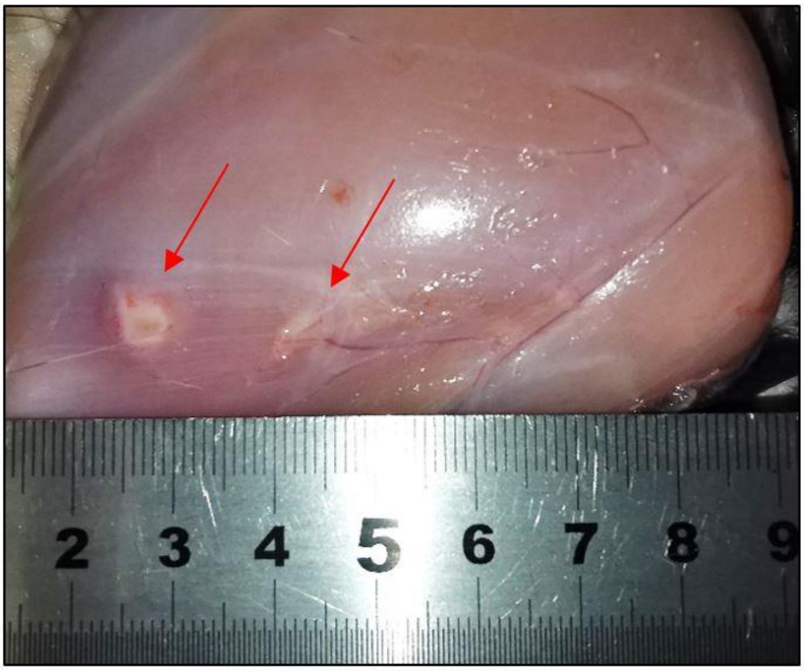

Figure 5: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side). The red arrows indicate the formed lesions.

One lesion was observed on the inner side of the left thigh above the skin as shown in Figure 6 while no lesions were observed below the skin as shown in Figure 7.

Figure 6: Macroscopic appearance of the discrete lesions from the inner side of the left thigh and above the skin. The red arrows indicate the formed lesions on the skin.

Figure 7: Macroscopic appearance of the inner side of the left thigh. No lesions were observed.

The muscle of the left thigh was dissected and one lesion was observed as shown in Figure 8.

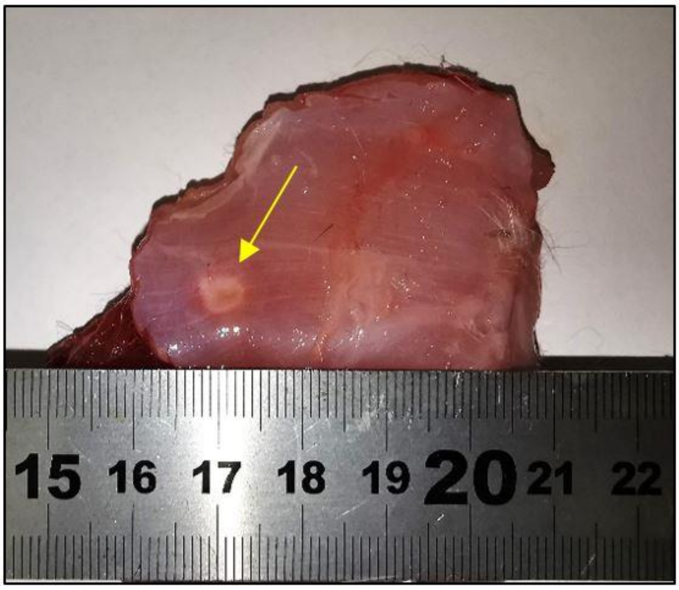

Figure 8: Macroscopic appearance of the formed lesion (indicated with yellow arrow) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The diameter and length of the formed lesion was 6 mm and 9 mm, respectively. Figure 9 demonstrates the dissection of the lesion that was created on the left thigh. This lesion was possibly created by a 30 s sonication time.

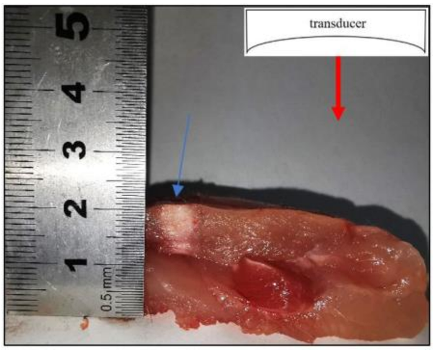

Figure 9: In situ dissection of the formed lesion (30 s sonication) of the left thigh. The blue arrow indicates the formed lesion and the red arrow indicates the beam direction.

Two discrete lesions were observed from the side of the sonication (outer side) on the right thigh above the skin and three discrete lesions below the skin as shown in Figure 10 and Figure 11, respectively.

Figure 10: The outer side of the right thigh above the skin after the treatment. The red arrows indicate the formed lesions.

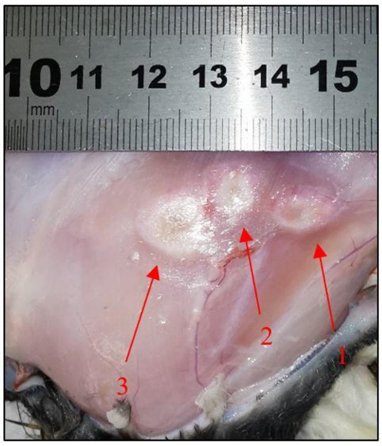

Figure 11: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed. The red arrows indicate the formed lesions.

One lesion was appeared on the inner side of the right thigh above and below the skin as shown in Figure 12 and Figure 13, respectively.

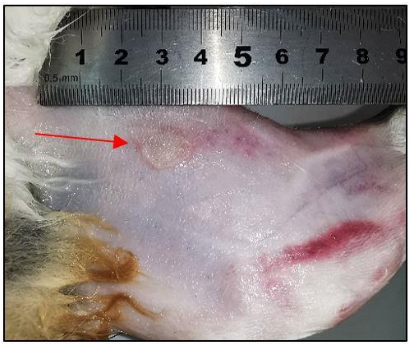

Figure 12: Macroscopic appearance of the lesion as observed from the inner side of the right thigh and above the skin. The red arrow indicates the lesion that was observed on the skin.

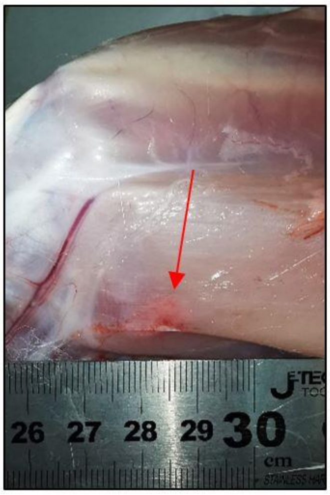

Figure 13: Macroscopic appearance of the lesion on the exposed muscle from the inner side of the right thigh. The red arrow indicates the formed lesion that was possibly created by a sonication of 30 s.

The muscle of the right thigh was dissected and three discrete lesions were observed as shown in Figure 14.

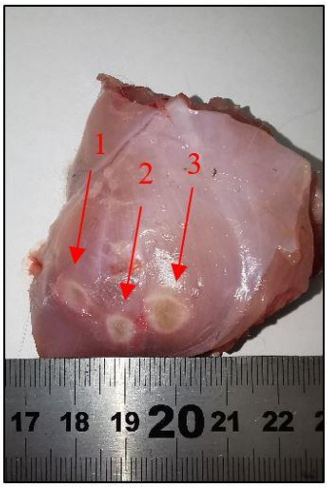

Figure 14: Macroscopic appearance of the formed lesions (indicated with red arrows) on the dissected muscle of the inner side of the right thigh (plane perpendicular to the beam).

Figure 15 demonstrates the vertical cross middle dissection of the lesion 2 and 3 that were created on the right thigh. Figure 16 demonstrates the vertical cross middle dissection of the lesion 1.

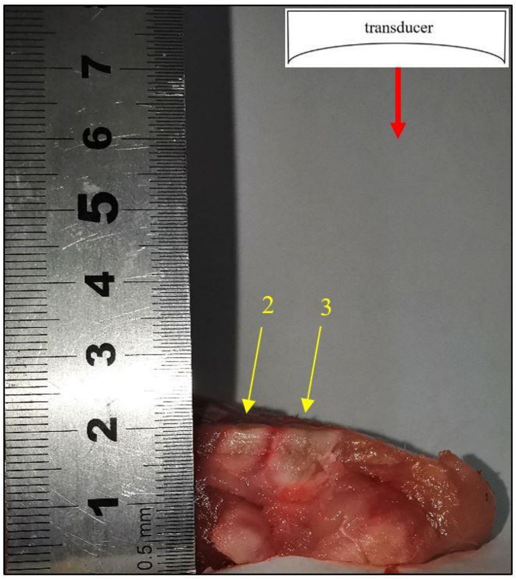

Figure 15: In situ dissection of the formed lesions 2 and 3 of the right thigh. The yellow arrows indicate the formed lesions and the red arrow indicates the beam direction.

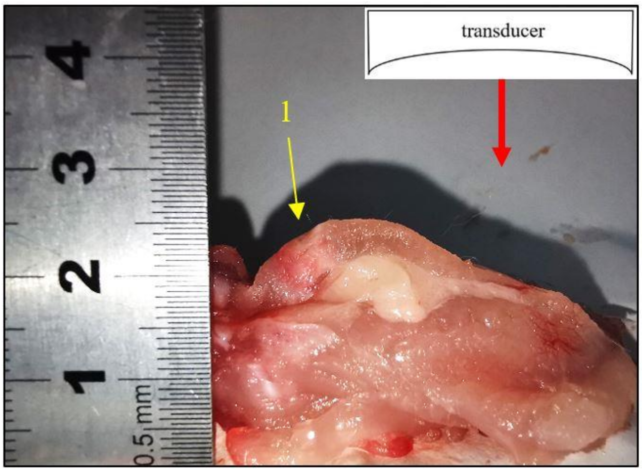

Figure 16: In situ dissection of the formed lesion 1 of the right thigh. The yellow arrow indicates the formed lesions and the red arrow indicates the beam direction.

Three (3) lesions were observed on the right thigh after the treatment (Table 3). The diameter and length of all lesions was measurable.

Table 3: Summary of results for the right thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	6	5
2	5	7
3	8	8

Table 4 lists the anesthesia record for the rabbit experiment.

Table 4: Anesthesia record for rabbit 23.

Animal ID			SOUNDPET_2020_23		
Species			Rabbit		
Sex			F		
Age			Adult		
Weight (Kg)			2.5		
Supplier			S. Ioannou Farm (CYCU5.05)		
Grant			SOUNDPET (INTEGRATED/0918/0008)		
Study			CY/EXP/PR.L01/2020		
Participants			Kyriakos Spanoudes		
			Theocharis Drakos		
			Nikolas Evripidou		
Principal Investigator			Christakis Damianou		
Time			**Anesthesia**		
13:30			Medetomidine 1.2 ml + Ketamine 0.5 ml		
13:45			Midazolam 0.5 ml		
Euthanasia			14:30 1 ml T-61		
Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
13:40	110	62	2	Absent	n/a
13:50	100	66	0	Absent	n/a
14:00	110	66	0	Absent	n/a
14:10	110	70	0	Absent	n/a
14:20	98	70	0	Absent	n/a

Rabbit 24

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	150
Acoustic power (W)	34.5
Sonication time (s)	5, 10, 20
Energy (J)	172.5, 345, 690
Cooling time (s)	60
Number of sonications	3
Step between sonications (mm)	15
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 3 (direction from rabbit feet to tail) at step movement of 15 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm.

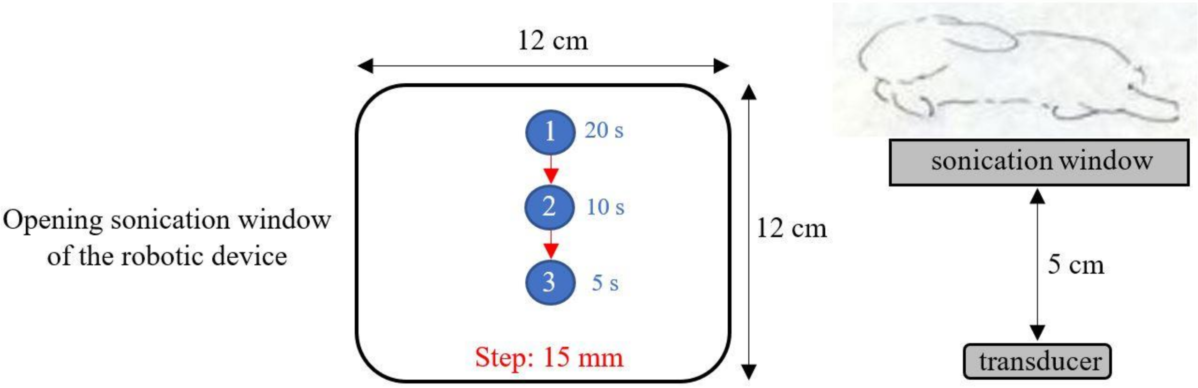

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	150
Acoustic power (W)	34.5
Sonication time (s)	5, 10, 20
Energy (J)	172.5, 345, 690
Cooling time (s)	60
Number of sonications	3
Step between sonications (mm)	15
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved from point 1 to 3 (direction from rabbit feet to tail) at step movement of 15 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm.

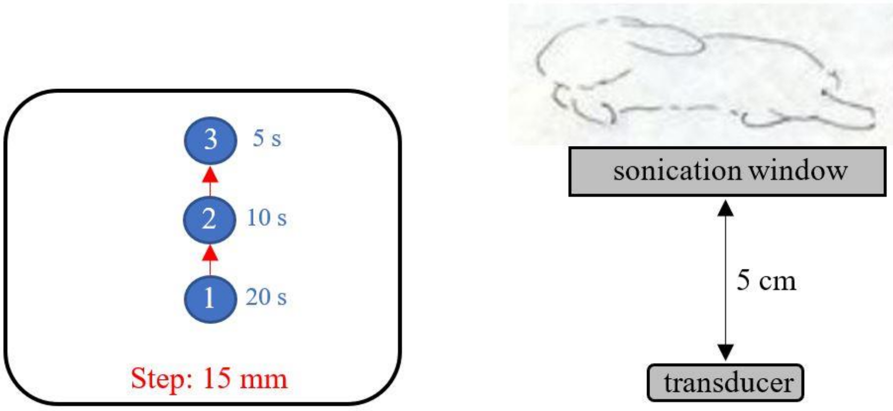

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory, that was used for the rabbit thigh ablations.

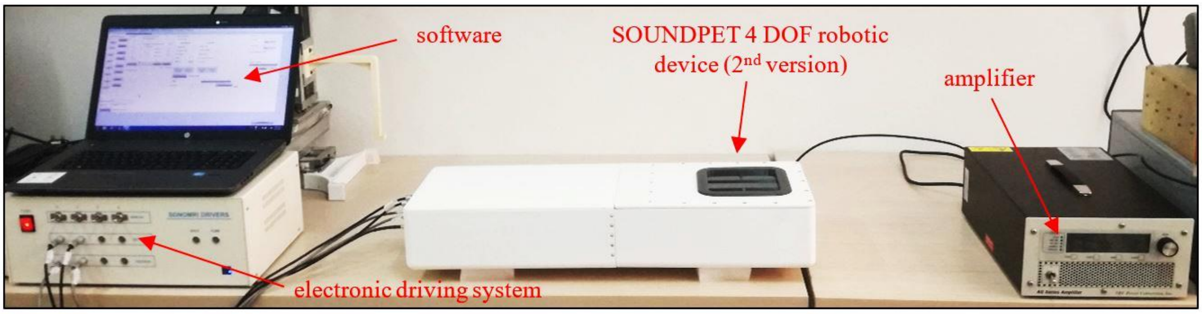

Figure 3: Experimental set-up for rabbit thigh ablation.

Results

No lesions were appeared on the outer side (sonication side) of the left thigh above and below the skin as shown in Figure 4 and Figure 5, respectively.

Figure 4: The outer side of the left thigh above the skin after the treatment.

Figure 5: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side).

One lesion was observed on the inner side of the left thigh above the skin as shown in Figure 6 while no lesions were observed below the skin as shown in Figure 7.

Figure 6: Macroscopic appearance of the lesion from the inner side of the left thigh and above the skin. The red arrow indicates the only formed lesion on the skin.

Figure 7: Macroscopic appearance of the inner side of the left thigh. No lesions were observed.

The muscle of the left thigh was dissected and no lesions were observed as shown in Figure 8.

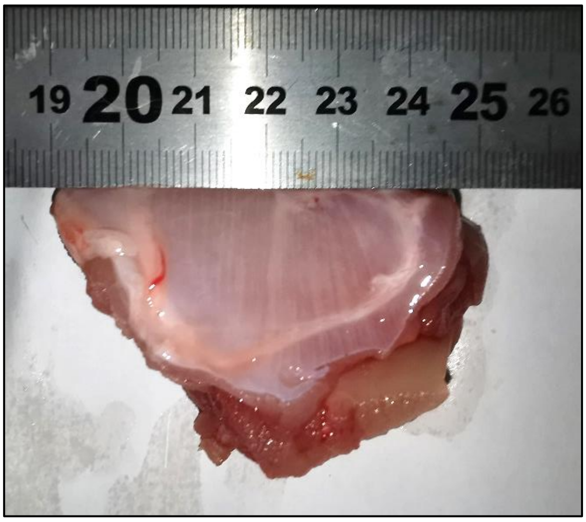

Figure 8: Macroscopic appearance of the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam). No lesions were observed.

One lesion was observed after the cross section of the exposed muscle of the left thigh as shown in Figure 9. The diameter and length of the lesion was 4 mm and 2 mm, respectively.

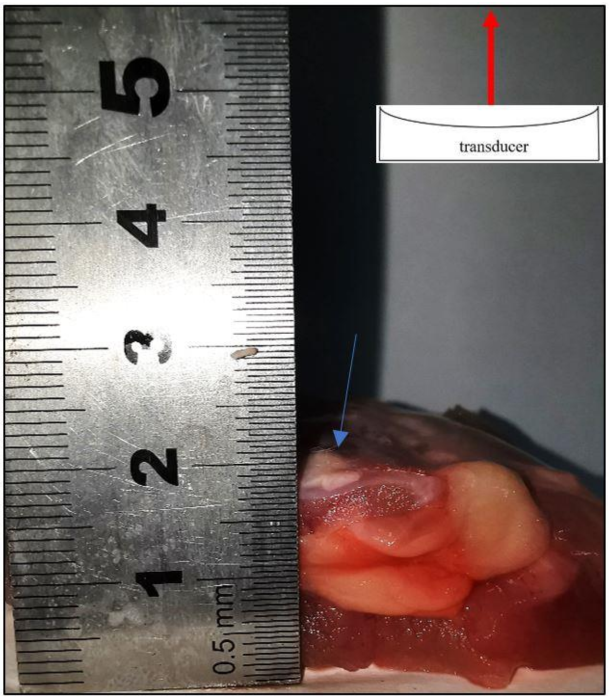

Figure 9: In situ dissection of the formed lesion that was appeared after the cross section of the exposed muscle of the left thigh. The blue arrow indicates the formed lesion and the red arrow indicates the beam direction.

No lesions were observed from the side of the sonication (outer side) on the right thigh above the skin and two discrete lesions were observed below the skin as shown in Figure 10 and Figure 11, respectively.

Figure 10: The outer side of the right thigh above the skin after the treatment. The red arrows indicate the formed lesions.

Figure 11: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed. The yellow arrows indicate the formed lesions.

No lesions were appeared on the inner side of the right thigh above and below the skin as shown in Figure 12 and Figure 13, respectively.

Figure 12: Macroscopic appearance of the inner side of the right thigh and above the skin. No lesions were observed on the skin.

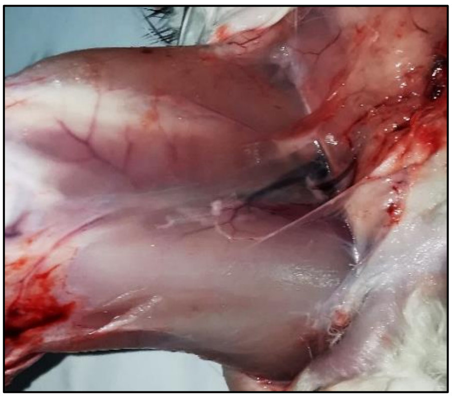

Figure 13: Macroscopic appearance of the exposed muscle from the inner side of the right thigh. No lesions were observed.

The muscle of the right thigh was dissected and two discrete lesions were observed as shown in Figure 14.

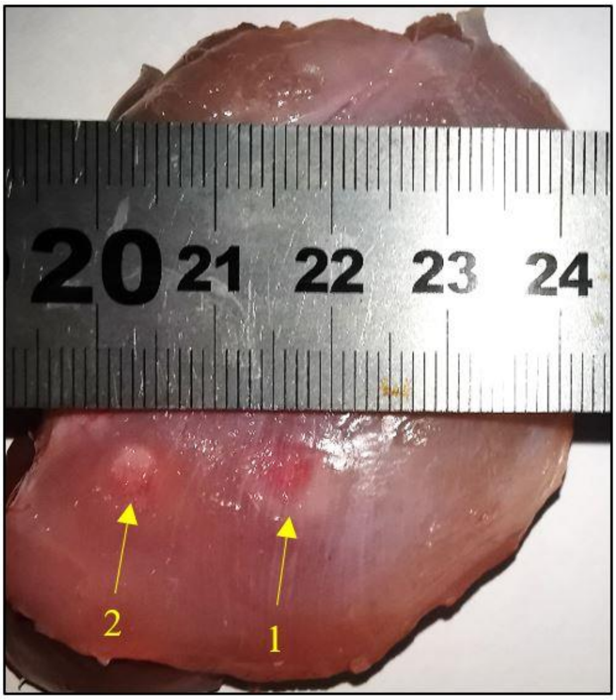

Figure 14: Macroscopic appearance of the formed lesions (indicated with yellow arrows) on the dissected muscle of the inner side of the right thigh (plane perpendicular to the beam).

Figure 15 demonstrates the vertical cross middle dissection of the lesions that were created on the right thigh.

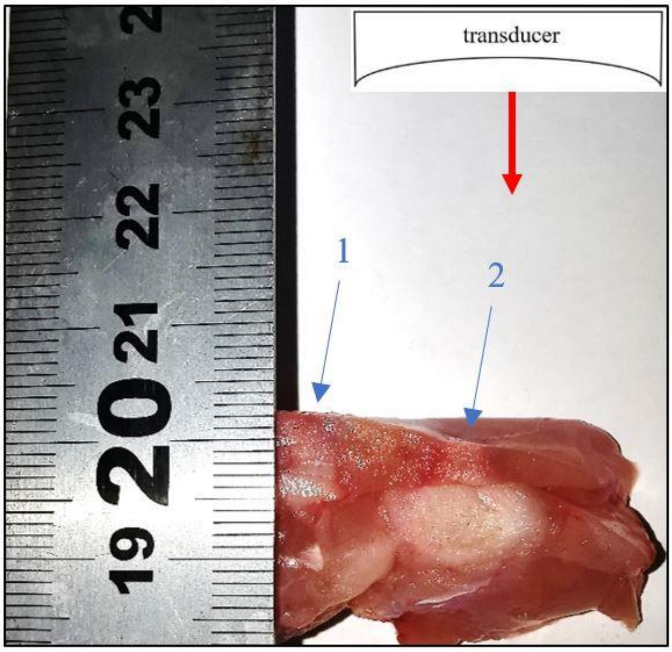

Figure 15: In situ dissection of the formed lesions of the right thigh. The blue arrows indicate the formed lesions and the red arrow indicates the beam direction.

Two (2) lesions were observed on the right thigh after the treatment (Table 3). The diameter and length of the lesions was measurable.

Table 3: Summary of results for the right thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	4	8
2	3	4
3	-	-

Table 4 lists the anesthesia record for the rabbit experiment.

Table 4: Anesthesia record for rabbit 24.

<table>
<tr><td colspan="3">Animal ID</td><td colspan="3">SOUNDPET_2020_24</td></tr>
<tr><td colspan="3">Species</td><td colspan="3">Rabbit</td></tr>
<tr><td colspan="3">Sex</td><td colspan="3">F</td></tr>
<tr><td colspan="3">Age</td><td colspan="3">Adult</td></tr>
<tr><td colspan="3">Weight (Kg)</td><td colspan="3">2.5</td></tr>
<tr><td colspan="3">Supplier</td><td colspan="3">S. Ioannou Farm (CYCU5.05)</td></tr>
<tr><td colspan="3">Grant</td><td colspan="3">SOUNDPET (INTEGRATED/0918/0008)</td></tr>
<tr><td colspan="3">Study</td><td colspan="3">CY/EXP/PR.L01/2020</td></tr>
<tr><td colspan="3" rowspan="3">Participants</td><td colspan="3">Kyriakos Spanoudes</td></tr>
<tr><td colspan="3">Theocharis Drakos</td></tr>
<tr><td colspan="3">Nikolas Evripidou</td></tr>
<tr><td colspan="3">Principal Investigator</td><td colspan="3">Christakis Damianou</td></tr>
<tr><td colspan="6"></td></tr>
<tr><td colspan="3">Time</td><td colspan="3">Anesthesia</td></tr>
<tr><td colspan="3">14:40</td><td colspan="3">Medetomidine 1.5 ml + Ketamine 0.5 ml</td></tr>
<tr><td colspan="3">14:50</td><td colspan="3">Midazolam 0.5 ml</td></tr>
<tr><td colspan="3">Euthanasia</td><td colspan="3">15:45 1 ml T-61</td></tr>
<tr><td colspan="6"></td></tr>
<tr><td>Time</td><td>Heart Rate</td><td>Resp. Rate</td><td>Urination
(0 to 3)</td><td>Absence of Movement /Pedal Reflex</td><td>Temp. (°C)</td></tr>
<tr><td>14:50</td><td>100</td><td>62</td><td>0</td><td>Absent</td><td>n/a</td></tr>
<tr><td>15:00</td><td>100</td><td>62</td><td>0</td><td>Absent</td><td>n/a</td></tr>
<tr><td>15:10</td><td>92</td><td>66</td><td>0</td><td>Absent</td><td>n/a</td></tr>
<tr><td>15:20</td><td>96</td><td>66</td><td>0</td><td>Absent</td><td>n/a</td></tr>
<tr><td>15:30</td><td>92</td><td>62</td><td>2</td><td>Absent</td><td>n/a</td></tr>
</table>

Rabbit 25

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	150
Acoustic power (W)	34.5
Sonication time (s)	10, 20, 30
Energy (J)	345, 690, 1035
Cooling time (s)	60
Number of sonications	3
Step between sonications (mm)	15
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 3 (direction from rabbit feet to tail) at step movement of 15 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm.

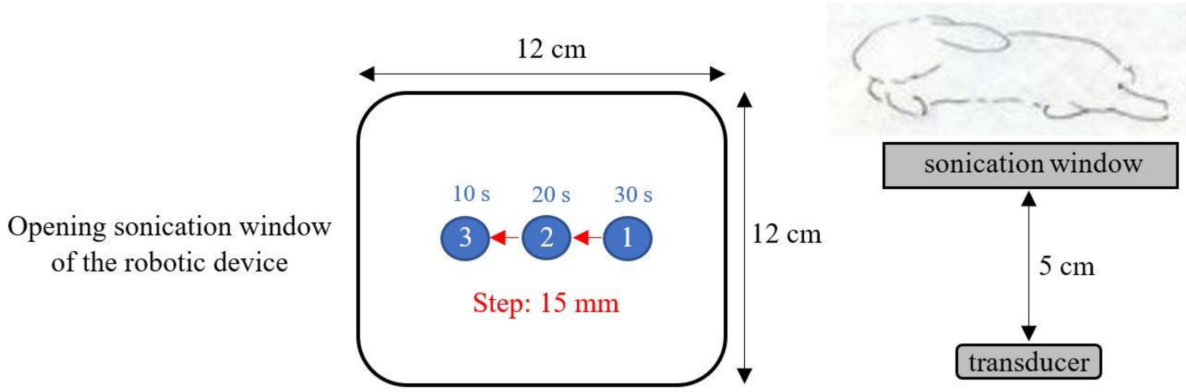

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	150
Acoustic power (W)	34.5
Sonication time (s)	10, 20, 30
Energy (J)	345, 690, 1035
Cooling time (s)	60
Number of sonications	3
Step between sonications (mm)	15
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved from point 1 to 3 (direction from rabbit tail to feet) at step movement of 15 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm.

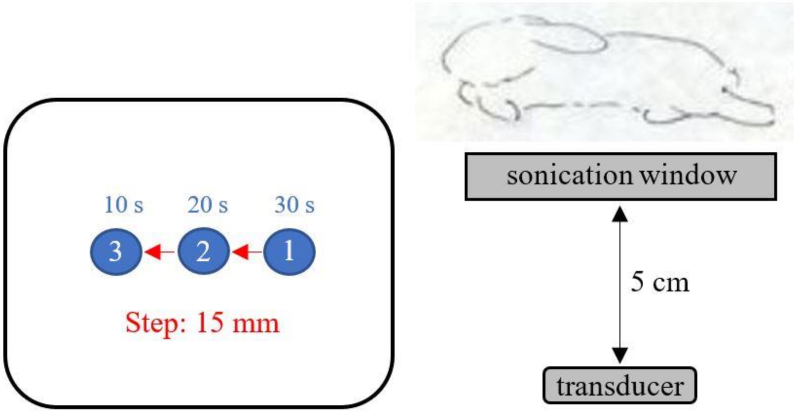

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory, that was used for the rabbit thigh ablations.

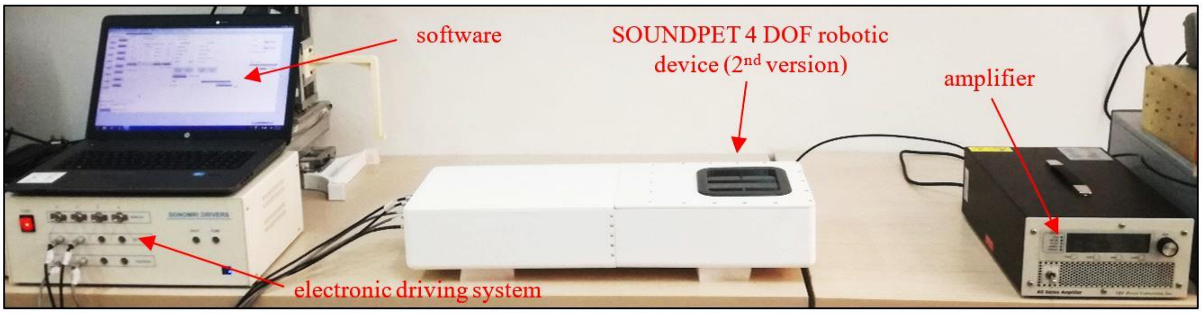

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

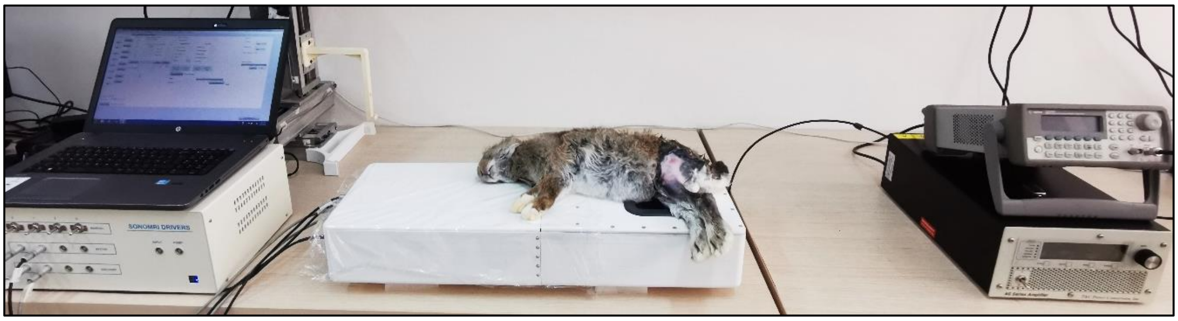

Figure 4: Animal positioning for the experiment.

Results

As shown in Figure 5, no lesions were appeared on the outer side (sonication side) of the left thigh above the skin while two discrete lesions were observed below the skin as shown in Figure 6.

Figure 5: The outer side of the left thigh above the skin after the treatment (electric power 150 W for 30 s, 20 s, 10 s).

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side). The red arrows indicate the formed lesions (electric power 150 W for 30 s, 20 s, 10 s).

No lesions were observed on the inner side of the left thigh above the skin as shown in Figure 7 while one lesion was observed below the skin as shown in Figure 8.

Figure 7: Macroscopic appearance of the inner side of the left thigh and above the skin. No lesions were observed (electric power 150 W for 30 s, 20 s, 10 s).

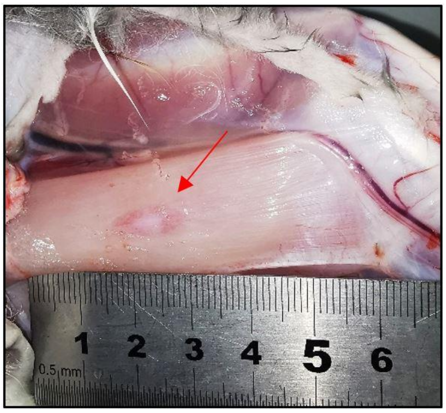

Figure 8: Macroscopic appearance of the inner side of the left thigh (electric power 150 W for 30 s, 20 s, 10 s). The red arrows indicates the formed lesion that was possibly created by 30 s sonication.

The muscle of the left thigh was dissected and one lesion was observed as shown in Figure 9.

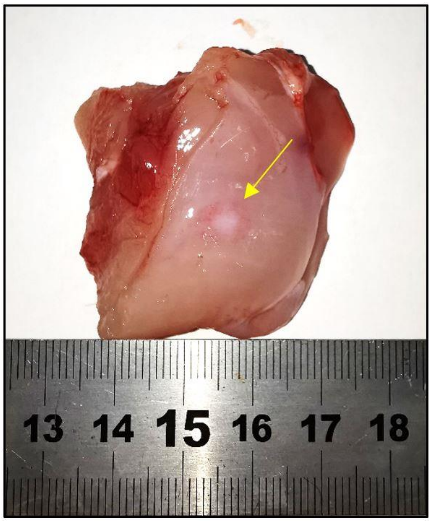

Figure 9: Macroscopic appearance of the formed lesion (indicated with yellow arrow, electric power 150 W for 30 s, 20 s, 10 s) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The diameter and length of the formed lesion was 6 mm and 2 mm, respectively. Figure 10 demonstrates the dissection of the lesion that was created on the left thigh. This lesion was possibly created by a 30 s sonication time.

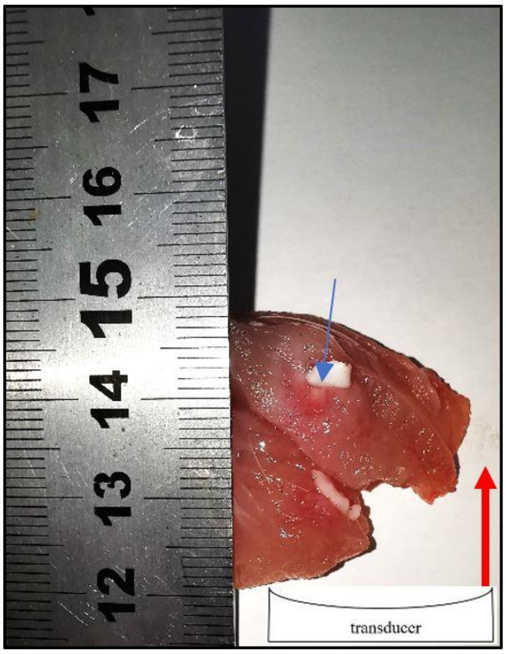

Figure 10: In situ dissection of the formed lesion (30 s sonication) of the left thigh. The blue arrow indicates the formed lesion and the red arrow indicates the beam direction (electric power 150 W for 30 s, 20 s, 10 s).

Two discrete lesions were observed from the side of the sonication (outer side) on the right thigh above and below the skin as shown in Figure 11 and Figure 12, respectively.

Figure 11: The outer side of the right thigh above the skin after the treatment. The red arrows indicate the formed lesions (electric power 150 W for 30 s, 20 s, 10 s).

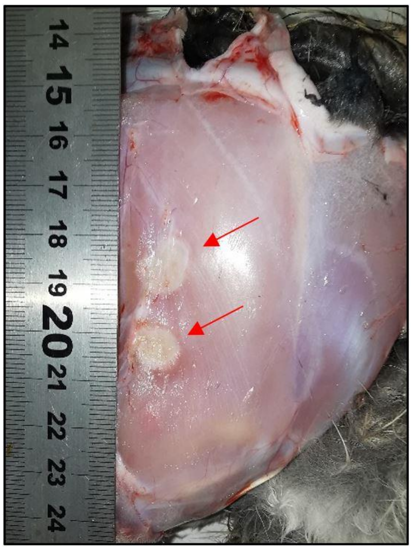

Figure 12: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed. The red arrows indicate the formed lesions (electric power 150 W for 30 s, 20 s, 10 s).

No lesions were appeared on the inner side of the right thigh above and below the skin as shown in Figure 13 and Figure 14, respectively.

Figure 13: Macroscopic appearance of the inner side of the right thigh and above the skin. No lesions were observed (electric power 150 W for 30 s, 20 s, 10 s).

Figure 14: Macroscopic appearance of the exposed muscle from the inner side of the right thigh. No lesions were observed (electric power 150 W for 30 s, 20 s, 10 s).

The muscle of the right thigh was dissected and two discrete lesions were observed as shown in Figure 15.

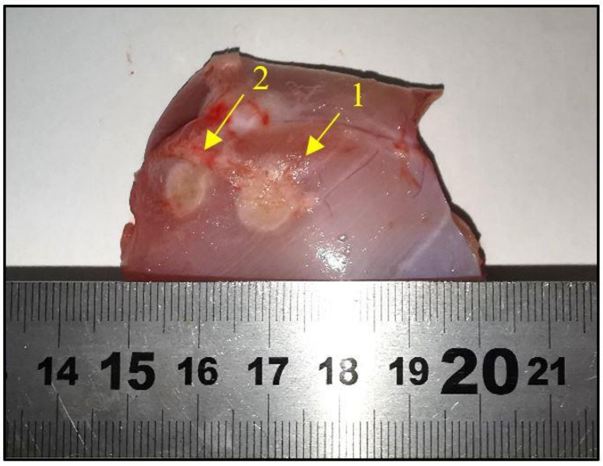

Figure 15: Macroscopic appearance of the formed lesions (indicated with yellow arrows, electric power 150 W for 30 s, 20 s, 10 s) on the dissected muscle of the inner side of the right thigh (plane perpendicular to the beam). Lesion 1 was for 30 s sonication and lesion 2 for 20 s sonication.

Figure 16 demonstrates the vertical cross middle dissection (with its mirror view) of the two lesions that were created on the right thigh.

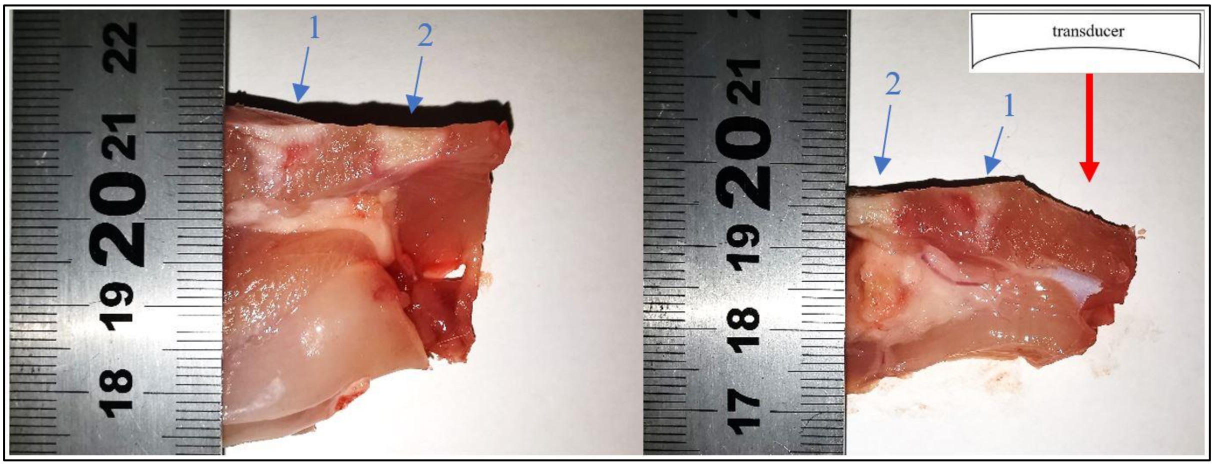

Figure 16: In situ dissection of the formed lesions of the right thigh. The blue arrows indicate the formed lesions and the red arrow indicates the beam direction (electric power 150 W for 30 s, 20 s, 10 s). Lesion 1 was for 30 s sonication and lesion 2 for 20 s sonication.

Two (2) lesions were observed on the right thigh after the treatment (Table 3). The diameter and length of the 2 lesions was measurable.

Table 3: Summary of results for the right thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	6	9
2	9	5
3	-	-

Table 4 lists the anesthesia record for the rabbit experiment.

Table 4: Anesthesia record for rabbit 25.

Animal ID	SOUNDPET_2020_25
Species	Rabbit
Sex	M
Age	Adult
Weight (Kg)	2.1
Supplier	S. Ioannou Farm (CYCU5.05)
Grant	SOUNDPET (INTEGRATED/0918/0008)
Study	CY/EXP/PR.L01/2020
Participants	Kyriakos Spanoudes
	Theocharis Drakos
	Nikolas Evripidou
Principal Investigator	Christakis Damianou
Time	**Anesthesia**
09:45	Medetomidine 1 ml + Ketamine 0.3 ml
09:55	Midazolam 0.5 ml
Euthanasia	10:20 1 ml T-61

Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
09:50	100	68	0	Absent	n/a
10:00	110	68	2	Absent	n/a
10:00	110	72	0	Absent	n/a
10:10	110	76	0	Absent	n/a
10:15	100	72	0	Absent	n/a

Rabbit 26

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	200
Acoustic power (W)	46
Sonication time (s)	5, 10, 20
Energy (J)	230, 460, 920
Cooling time (s)	60
Number of sonications	3
Step between sonications (mm)	15
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 3 (direction from rabbit feet to tail) at step movement of 15 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm.

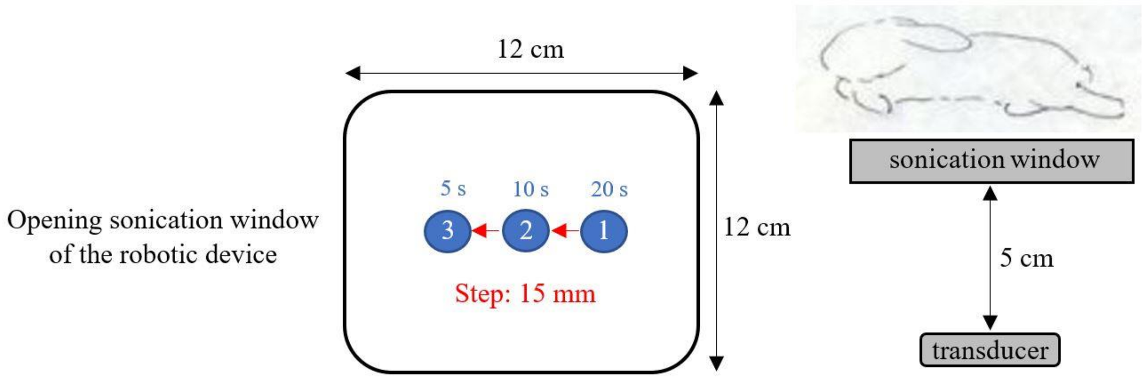

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	200
Acoustic power (W)	46
Sonication time (s)	5, 10, 20
Energy (J)	230, 460, 920
Cooling time (s)	60
Number of sonications	3
Step between sonications (mm)	15
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved from point 1 to 3 (direction from rabbit tail to feet) at step movement of 15 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm.

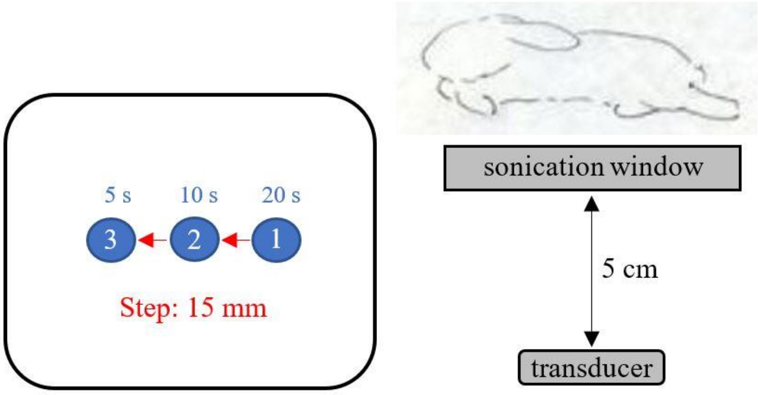

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory, that was used for the rabbit thigh ablations.

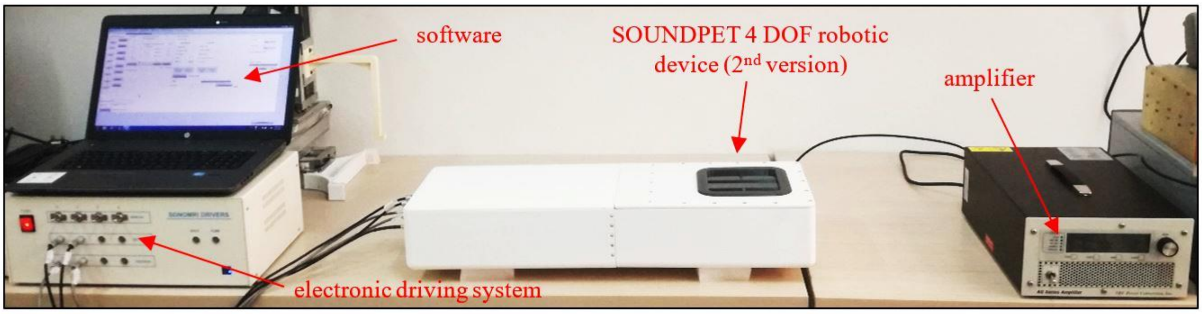

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

Figure 4: Animal positioning for the experiment.

Results

As shown in Figure 5, two lesions were appeared on the outer side (sonication side) of the left thigh above the skin while all discrete lesions were visible below the skin as shown in Figure 6.

Figure 5: The outer side of the left thigh above the skin after the treatment. The yellow arrows indicate the formed lesions (electric power 200 W for 20 s, 10 s, 5 s).

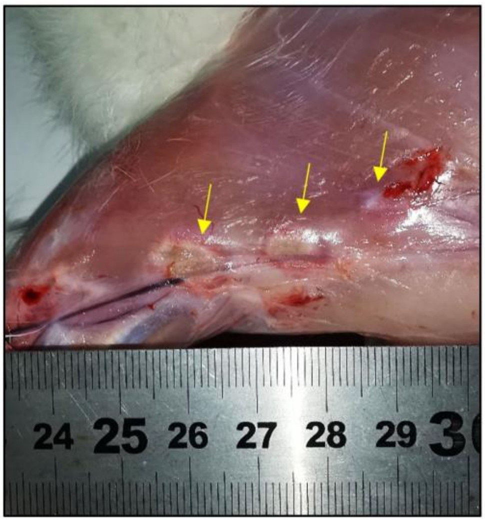

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side). The yellow arrows indicate the formed lesions (electric power 200 W for 20 s, 10 s, 5 s).

One lesion was observed on the inner side of the left thigh above and below the skin as shown in Figure 7 and Figure 8, respectively.

Figure 7: Macroscopic appearance of the only formed lesion that was observed on the inner side of the left thigh and above the skin. The yellow arrow indicates the formed lesion on the skin (electric power 200 W for 20 s, 10 s, 5 s).

Figure 8: Macroscopic appearance of the inner side of the left thigh. The yellow arrow indicates the formed lesion below the skin (electric power 200 W for 20 s, 10 s, 5 s).

The muscle of the left thigh was dissected and three lesions were observed as shown in Figure 9.

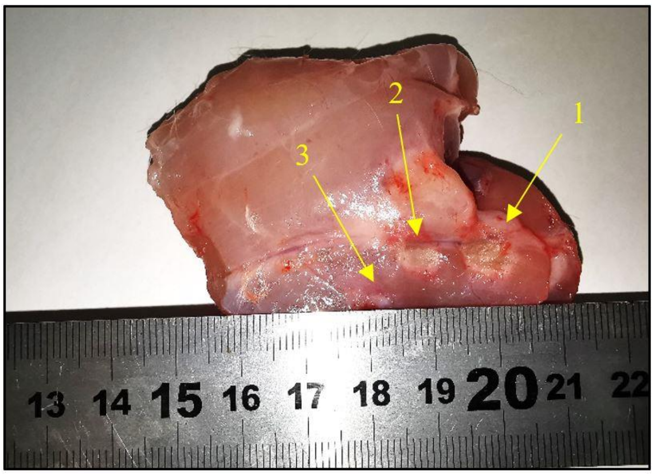

Figure 9: Macroscopic appearance of the formed lesions (indicated with yellow arrows, (electric power 200 W for 20 s, 10 s, 5 s) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam). Lesion 1 was for 20 s sonication, lesion 2 for 10 s and lesion 3 for 5 s.

The diameter of the formed lesions was 8 mm, 7 mm, and 3 mm for lesion 1, 2, and 3 respectively. Figure 10 demonstrates the dissection of the lesions 1 and 2 that were created on the left thigh and Figure 11 shows the dissection of the lesion 3.

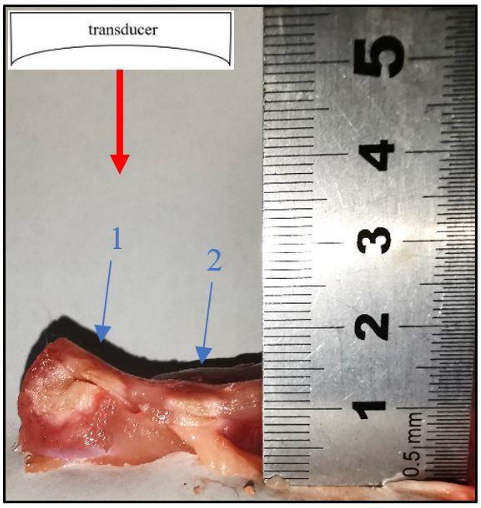

Figure 10: In situ dissection of the formed lesions 1 and 2 of the left thigh. The blue arrows indicate the formed lesions and the red arrow indicates the beam direction (electric power 200 W for 20 s, 10 s). Lesion 1 was for 20 s sonication and lesion 2 for 10 s.

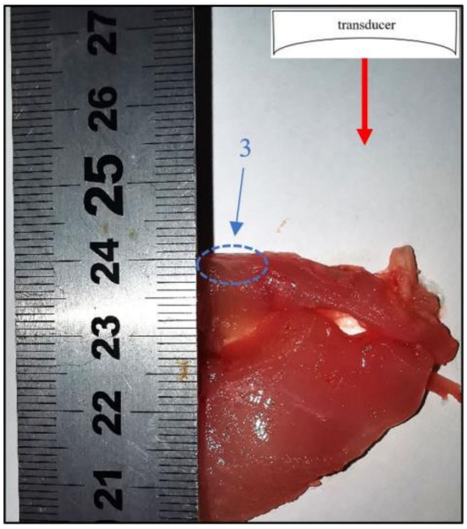

Figure 11: In situ dissection of the formed lesion 3 (electric power 200 W for 5 s) of the left thigh. The blue arrow indicates the formed lesion which was superficial and the red arrow indicates the beam direction. Lesion 3 was for 5 s sonication.

Three (3) lesions were observed on the right thigh after the treatment (Table 3). The diameter and length of the 2 lesions was measurable while the other lesion was superficial.

Table 3: Summary of results for the right thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	8	8
2	7	4
3	3	-

Two discrete lesions were observed from the side of the sonication (outer side) on the right thigh above the skin and three discrete lesions were observed below the skin as shown in Figure 12 and Figure 13, respectively.

Figure 12: The outer side of the right thigh above the skin after the treatment. The yellow arrows indicate the formed lesions (electric power 200 W for 20 s, 10 s, 5 s).

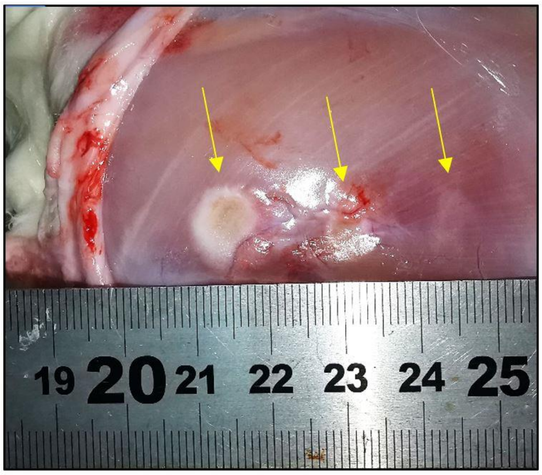

Figure 13: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed. The yellow arrows indicate the formed lesions (electric power 200 W for 20 s, 10 s, 5 s).

Two lesions were appeared on the inner side of the right thigh above the skin while one lesion was observed below the skin as shown in Figure 14 and Figure 15, respectively.

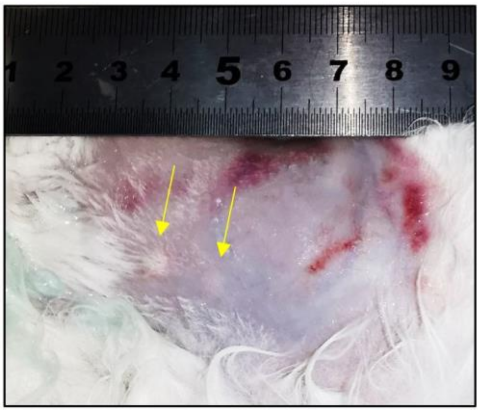

Figure 14: Macroscopic appearance of the lesions as observed from the inner side of the right thigh and above the skin. The yellow arrows indicate the lesions that were observed on the skin (electric power 200 W for 20 s, 10 s, 5 s).

Figure 15: Macroscopic appearance of the lesion on the exposed muscle from the inner side of the right thigh. The yellow arrow indicates the formed lesion that was created by a sonication of 20 s (electric power 200 W).

The muscle of the right thigh was dissected and two discrete lesions were observed as shown in Figure 16.

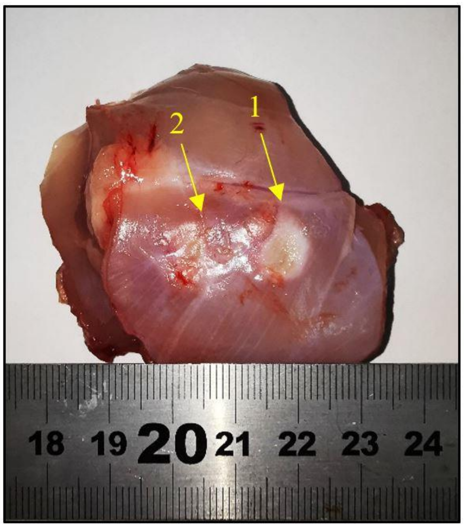

Figure 16: Macroscopic appearance of the formed lesions (indicated with yellow arrows, electric power 200 W for 20 s, 10 s, 5 s) on the dissected muscle of the inner side of the right thigh (plane perpendicular to the beam). Lesion 1 was for 20 s sonication and lesion 2 for 10 s.

Figure 17 demonstrates the vertical cross middle dissection of the lesions were created on the right thigh.

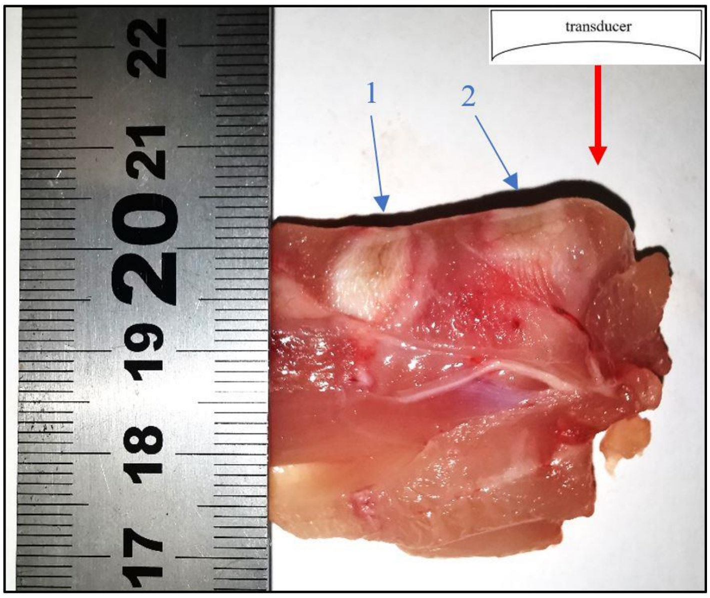

Figure 17: In situ dissection of the formed lesions 2 and 3 of the right thigh. The blue arrows indicate the formed lesions and the red arrow indicates the beam direction (electric power 200 W for 20 s, 10 s, 5 s). Lesion 1 was for 20 s sonication and lesion 2 for 10 s.

Two (2) lesions were observed on the right thigh after the treatment (Table 4). The diameter and length of the 2 lesions was measurable.

Table 4: Summary of results for the right thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	8	9
2	7	8
3	-	-

Table 5 lists the anesthesia record for the rabbit experiment.

Table 5: Anesthesia record for rabbit 26.

Animal ID	SOUNDPET_2020_26
Species	Rabbit
Sex	M
Age	Adult
Weight (Kg)	2.2
Supplier	S. Ioannou Farm (CYCU5.05)
Grant	SOUNDPET (INTEGRATED/0918/0008)
Study	CY/EXP/PR.L01/2020
Participants	Kyriakos Spanoudes
	Theocharis Drakos
	Nikolas Evripidou

Principal Investigator	Christakis Damianou
Time	**Anesthesia**
10:30	Medetomidine 1 ml + Ketamine 0.3 ml
10:40	Midazolam 0.5 ml
Euthanasia	11:20 1 ml T-61

Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
10:30	100	62	0	Absent	n/a
10:40	100	72	0	Absent	n/a
10:50	110	68	0	Absent	n/a
11:00	100	62	1	Absent	n/a

Rabbit 27

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	200
Acoustic power (W)	46
Sonication time (s)	10
Energy (J)	460
Cooling time (s)	60
Number of sonications	3
Step between sonications (mm)	15
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 3 (direction from rabbit feet to tail) at step movement of 15 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm.

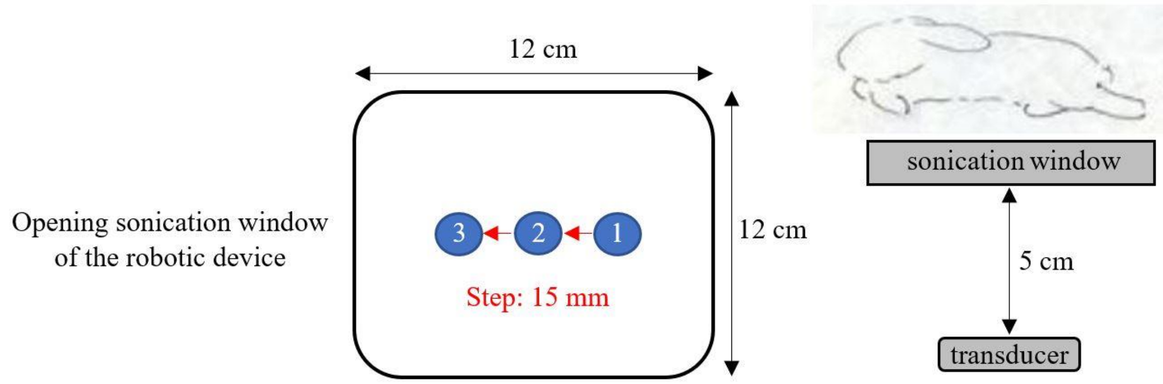

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	200
Acoustic power (W)	46
Sonication time (s)	5
Energy (J)	230
Cooling time (s)	60
Number of sonications	3
Step between sonications (mm)	15
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved from point 1 to 3 (direction from rabbit feet to tail) at step movement of 15 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm.

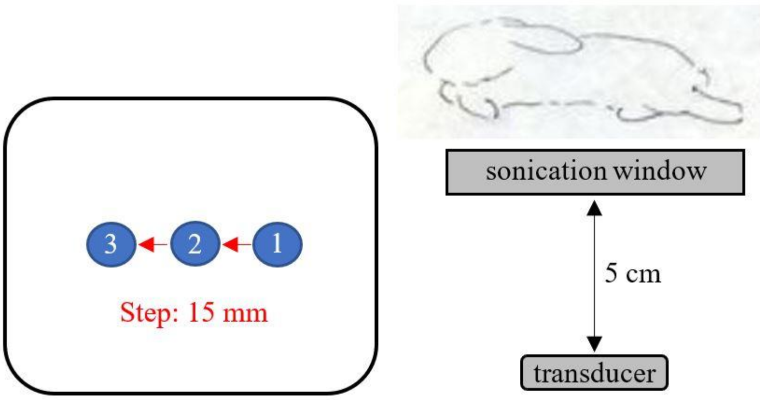

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory, that was used for the rabbit thigh ablations.

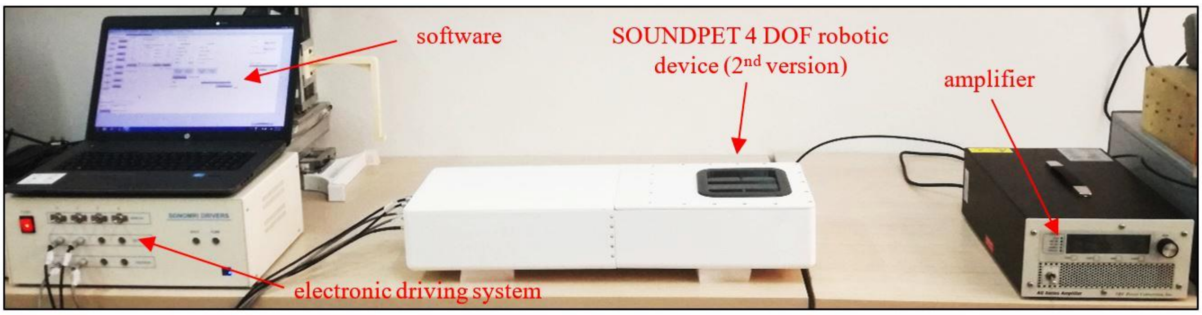

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

Figure 4: Animal positioning for the experiment.

Results

As shown in Figure 5, one lesion was appeared on the outer side (sonication side) of the left thigh above the skin while three discrete lesions were observed below the skin as shown in Figure 6.

Figure 5: The outer side of the left thigh above the skin after the treatment. The red arrow indicates the formed lesion (electric power 200 W for 10 s).

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side). The red arrows indicate the formed lesions (electric power 200 W for 10 s).

One lesion was observed on the inner side of the left thigh above the skin as shown in Figure 7 while no lesions were observed below the skin as shown in Figure 8.

Figure 7: Macroscopic appearance of the lesion that was appeared on the inner side of the left thigh and above the skin. The red arrow indicates the formed lesion on the skin (electric power 200 W for 10 s).

Figure 8: Macroscopic appearance of the inner side of the left thigh. No lesions were observed (electric power 200 W for 10 s).

The muscle of the left thigh was dissected and three lesions were observed as shown in Figure 9.

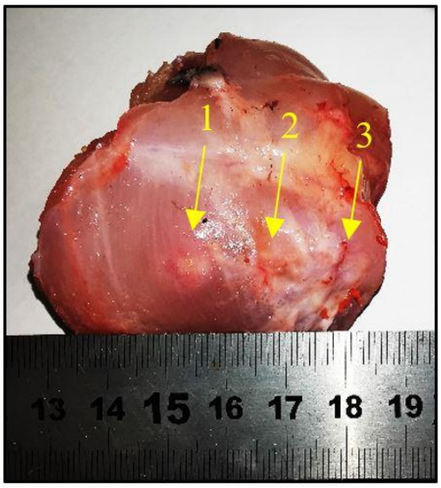

Figure 9: Macroscopic appearance of the formed lesions (indicated with yellow arrows, electric power 200 W for 10 s) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

Figure 10 demonstrates the vertical cross middle dissection of the lesions that were created on the left thigh.

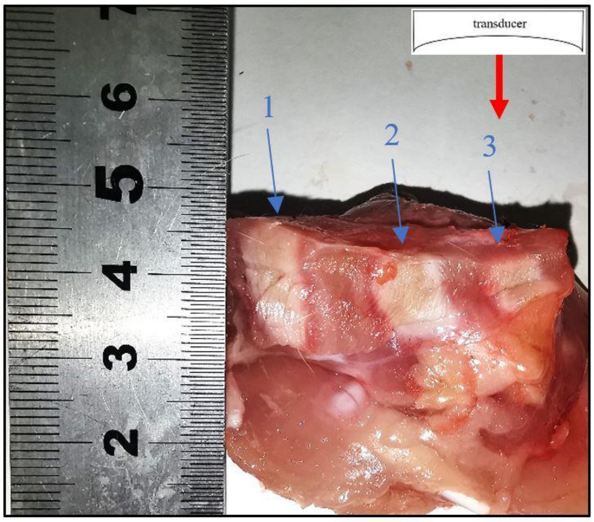

Figure 10: In situ dissection of the formed lesions of the left thigh. The blue arrows indicate the formed lesions and the red arrow indicates the beam direction (electric power 200 W for 10 s).

Three (3) lesions were observed on the right thigh after the treatment (Table 3). The diameter and length of the 3 lesions was measurable.

Table 3: Summary of results for the right thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	6	14
2	6	8
3	4	6

No lesions were observed from the side of the sonication (outer side) on the right thigh above and below the skin as shown in Figure 11 and Figure 12, respectively.

Figure 11: The outer side of the right thigh above the skin after the treatment. No lesions were observed (electric power 200 W for 5 s).

Figure 12: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed. No lesions were observed (electric power 200 W for 5 s).

No lesions were appeared on the inner side of the right thigh above and below the skin as shown in Figure 13 and Figure 14, respectively.

Figure 13: Macroscopic appearance of the inner side of the right thigh and above the skin. No lesions were observed (electric power 200 W for 5 s).

Figure 14: Macroscopic appearance of the exposed muscle from the inner side of the right thigh. No lesions were observed (electric power 200 W for 5 s).

The muscle of the right thigh was dissected and three discrete lesions (very faint on the surface) were observed as shown in Figure 15.

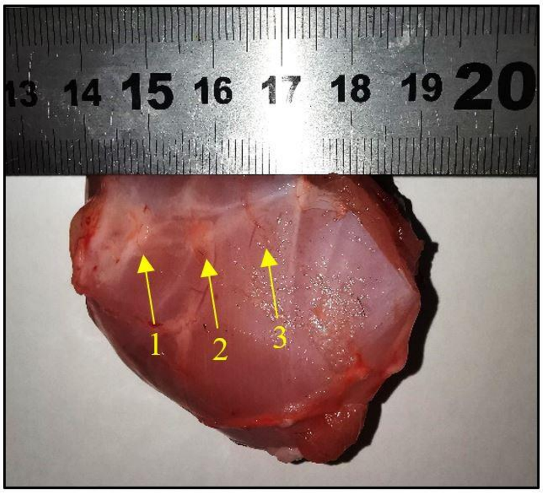

Figure 15: Macroscopic appearance of the formed lesions (indicated with yellow arrows, electric power 200 W for 5 s) on the dissected muscle of the inner side of the right thigh (plane perpendicular to the beam).

Figure 16 demonstrates the vertical cross middle dissection of the lesions that were created on the right thigh.

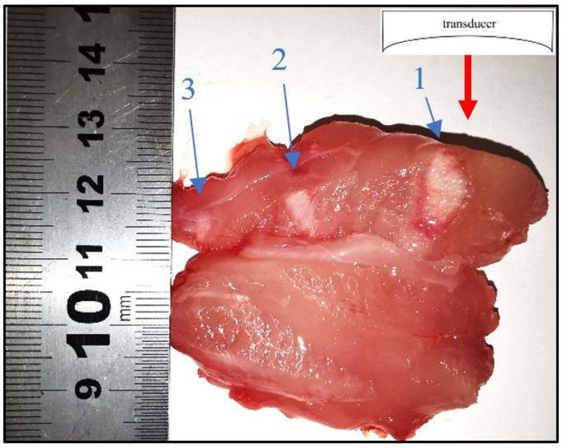

Figure 16: In situ dissection of the formed lesions created on the right thigh. The blue arrows indicate the formed lesions and the red arrow indicates the beam direction (electric power 200 W for 5 s).

Three (3) lesions were observed on the right thigh after the treatment (Table 4). The diameter and length of all lesions was measurable.

Table 4: Summary of results for the right thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	4	13
2	3	8
3	2	5

Table 5 lists the anesthesia record for the rabbit experiment.

Table 5: Anesthesia record for rabbit 27.

Animal ID	SOUNDPET_2020_27
Species	Rabbit
Sex	M
Age	Adult
Weight (Kg)	2.15
Supplier	S. Ioannou Farm (CYCU5.05)
Grant	SOUNDPET (INTEGRATED/0918/0008)
Study	CY/EXP/PR.L01/2020
Participants	Kyriakos Spanoudes
	Theocharis Drakos
	Nikolas Evripidou
Principal Investigator	Christakis Damianou
Time	**Anesthesia**
11:30	Medetomidine 1 ml + Ketamine 0.3 ml
11:35	Midazolam 0.5 ml
Euthanasia	12:30 1 ml T-61

Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
11:30	120	62	1	Absent	n/a
11:40	110	68	0	Absent	n/a
11:50	110	72	0	Absent	n/a
12:00	100	76	0	Absent	n/a
12:15	110	80	0	Absent	n/a
12:30	100	72	0	Absent	n/a

Rabbit 28

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	250
Acoustic power (W)	57.5
Sonication time (s)	5, 10, 20
Energy (J)	287.5, 575, 1150
Cooling time (s)	60
Number of sonications	3
Step between sonications (mm)	15
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 3 (direction from rabbit feet to tail) at step movement of 15 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm.

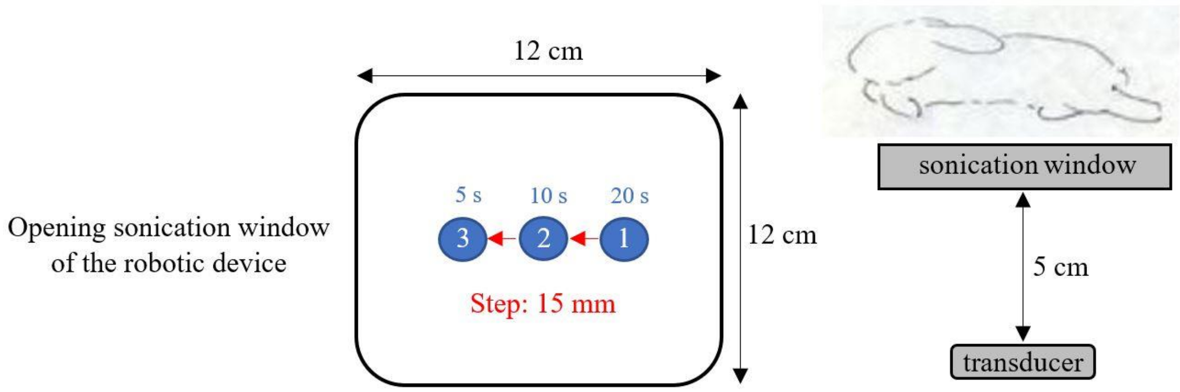

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	250
Acoustic power (W)	57.5
Sonication time (s)	5, 10, 20
Energy (J)	287.5, 575, 1150
Cooling time (s)	60
Number of sonications	3
Step between sonications (mm)	15
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved from point 1 to 3 (direction from rabbit tail to feet) at step movement of 15 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm.

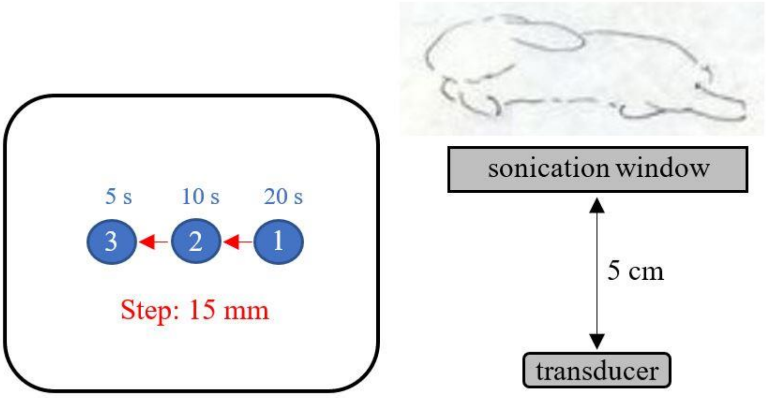

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory, that was used for the rabbit thigh ablations.

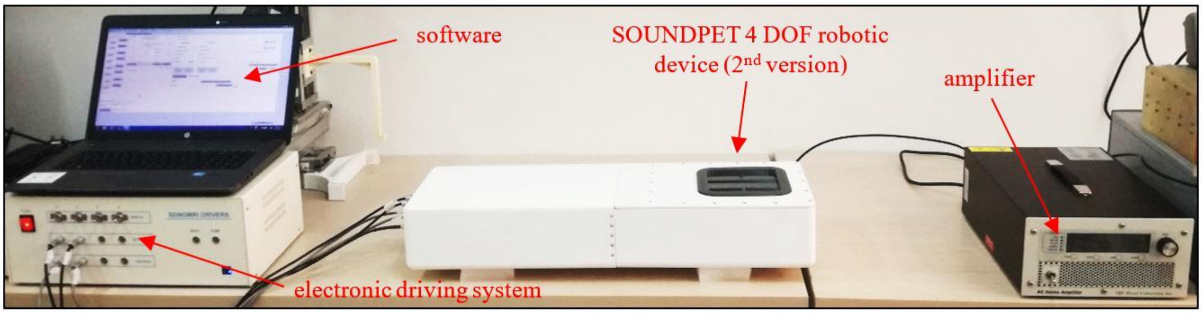

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

Figure 4: Animal positioning for the experiment.

Results

As shown in Figure 5, two discrete lesions were appeared on the outer side (sonication side) of the left thigh above the skin while three discrete lesions were observed below the skin as shown in Figure 6.

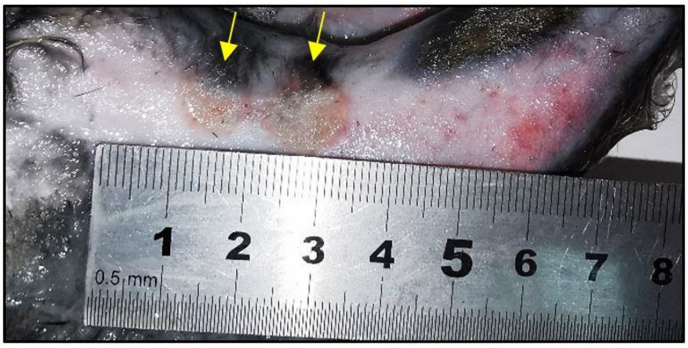

Figure 5: The outer side of the left thigh above the skin after the treatment. The yellow arrows indicate the formed lesions (electric power 250 W for 20 s, 10 s, 5 s).

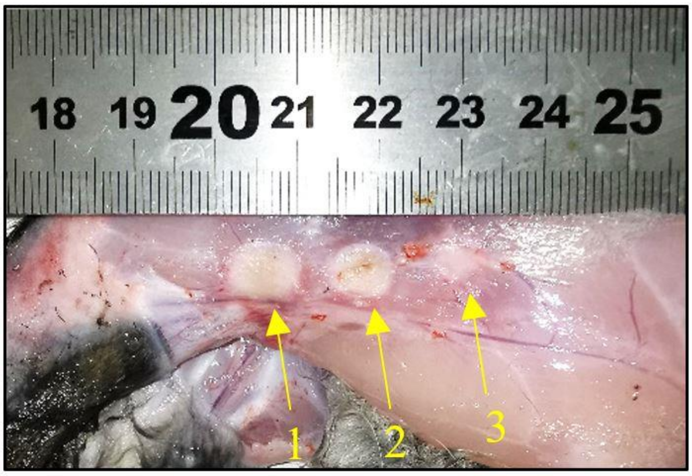

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side). The red arrows indicate the formed lesions (electric power 250 W for 20 s, 10 s, 5 s).

No lesions were observed on the inner side of the left thigh above and below the skin as shown in Figure 7 and Figure 8, respectively.

Figure 7: Macroscopic appearance of the inner side of the left thigh and above the skin. No lesions were observed (electric power 250 W for 20 s, 10 s, 5 s).

Figure 8: Macroscopic appearance of the inner side of the left thigh. No lesions were observed (electric power 250 W for 20 s, 10 s, 5 s).

The muscle of the left thigh was dissected and three lesions were observed as shown in Figure 9.

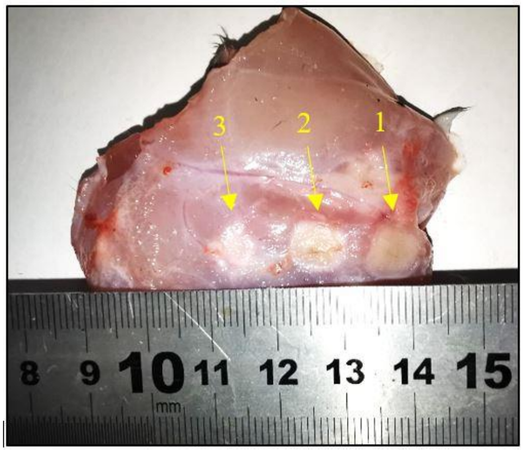

Figure 9: Macroscopic appearance of the formed lesions (indicated with yellow arrow, electric power 250 W for 20 s, 10 s, 5 s) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam). Lesion 1 was for 20 s sonication, lesion 2 for 10 s and lesion 3 for 5 s.

Figure 10 demonstrates the vertical cross middle dissection of the lesions that were created on the left thigh.

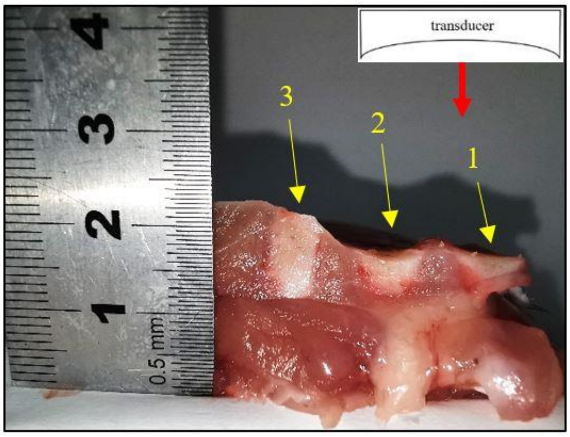

Figure 10: In situ dissection of the formed lesions of the left thigh. The yellow arrows indicate the formed lesions and the red arrow indicates the beam direction (electric power 250 W for 20 s, 10 s, 5 s). Lesion 1 was for 20 s sonication, lesion 2 for 10 s and lesion 3 for 5 s.

Three (3) lesions were observed on the left thigh after the treatment (Table 4). The diameter and length of all lesions was measurable.

Table 4: Summary of results for the left thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	9	3
2	8	4
3	7	10

Two discrete lesions were observed from the side of the sonication (outer side) on the right thigh above the skin and three discrete lesions below the skin as shown in Figure 11 and Figure 12, respectively.

Figure 11: The outer side of the right thigh above the skin after the treatment. The yellow arrows indicate the formed lesions (electric power 250 W for 20 s, 10 s, 5 s).

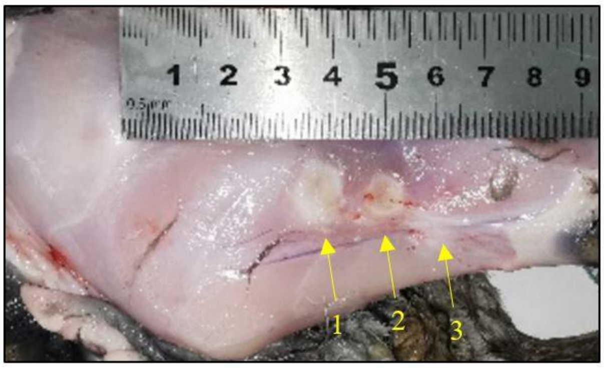

Figure 12: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed. The yellow arrows indicate the formed lesions (electric power 250 W for 20 s, 10 s, 5 s).

One lesion was appeared on the inner side of the right thigh above the skin as shown in Figure 13 and no lesions were observed below the skin as shown in Figure 14, respectively.

Figure 13: Macroscopic appearance of the lesion as observed from the inner side of the right thigh and above the skin. The red arrow indicates the lesion that was observed on the skin (electric power 250 W for 20 s, 10 s, 5 s).

Figure 14: Macroscopic appearance of the exposed muscle from the inner side of the right thigh. No lesions were observed (electric power 250 W for 20 s, 10 s, 5 s).

The muscle of the right thigh was dissected and three discrete lesions were observed as shown in Figure 15.

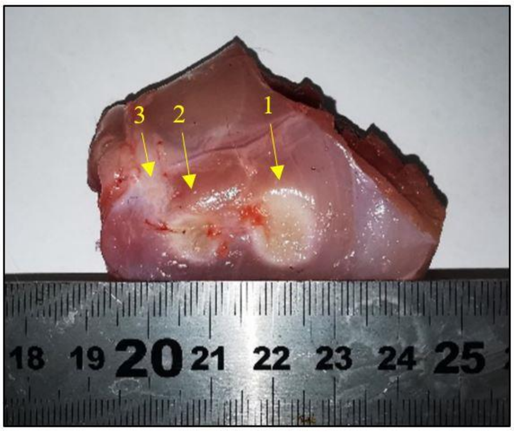

Figure 15: Macroscopic appearance of the formed lesions (indicated with red arrows, electric power 250 W for 20 s, 10 s, 5 s) on the dissected muscle of the inner side of the right thigh (plane perpendicular to the beam). Lesion 1 was for 20 s sonication, lesion 2 for 10 s and lesion 3 for 5 s.

Figure 16 demonstrates the vertical cross middle dissection of the lesions that were created on the right thigh.

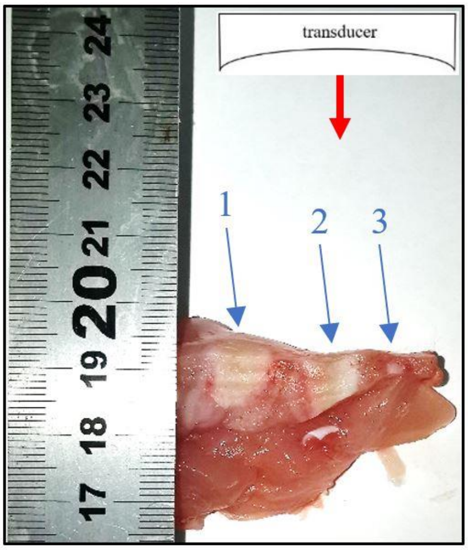

Figure 16: In situ dissection of the formed lesions of the right thigh. The blue arrows indicate the formed lesions and the red arrow indicates the beam direction (electric power 250 W for 20 s, 10 s, 5 s). Lesion 1 was for 20 s sonication, lesion 2 for 10 s and lesion 3 for 5 s.

Three (3) lesions were observed on the right thigh after the treatment (Table 3). The diameter and length of all lesions was measurable.

Table 3: Summary of results for the right thigh.

Lesion Number	Lesion Diameter (mm)	Lesion Length (mm)
1	11	8
2	7	7
3	2	2

Table 4 lists the anesthesia record for the rabbit experiment.

Table 4: Anesthesia record for rabbit 28.

<table>
<tr><td colspan="3">Animal ID</td><td colspan="3">SOUNDPET_2020_28</td></tr>
<tr><td colspan="3">Species</td><td colspan="3">Rabbit</td></tr>
<tr><td colspan="3">Sex</td><td colspan="3">M</td></tr>
<tr><td colspan="3">Age</td><td colspan="3">Adult</td></tr>
<tr><td colspan="3">Weight (Kg)</td><td colspan="3">2.15</td></tr>
<tr><td colspan="3">Supplier</td><td colspan="3">S. Ioannou Farm (CYCU5.05)</td></tr>
<tr><td colspan="3">Grant</td><td colspan="3">SOUNDPET (INTEGRATED/0918/0008)</td></tr>
<tr><td colspan="3">Study</td><td colspan="3">CY/EXP/PR.L01/2020</td></tr>
<tr><td colspan="3" rowspan="3">Participants</td><td colspan="3">Kyriakos Spanoudes</td></tr>
<tr><td colspan="3">Theocharis Drakos</td></tr>
<tr><td colspan="3">Nikolas Evripidou</td></tr>
<tr><td colspan="3">Principal Investigator</td><td colspan="3">Christakis Damianou</td></tr>
<tr><td colspan="6"></td></tr>
<tr><td colspan="3">Time</td><td colspan="3">Anesthesia</td></tr>
<tr><td colspan="3">13:30</td><td colspan="3">Medetomidine 1 ml + Ketamine 0.3 ml</td></tr>
<tr><td colspan="3">13:40</td><td colspan="3">Midazolam 0.5 ml</td></tr>
<tr><td colspan="3">Euthanasia</td><td colspan="3">14:00 1 ml T-61</td></tr>
<tr><td colspan="6"></td></tr>
<tr><td>Time</td><td>Heart Rate</td><td>Resp. Rate</td><td>Urination
(0 to 3)</td><td>Absence of Movement /Pedal Reflex</td><td>Temp. (°C)</td></tr>
<tr><td>13:30</td><td>120</td><td>78</td><td>0</td><td>Absent</td><td>n/a</td></tr>
<tr><td>13:40</td><td>120</td><td>76</td><td>1</td><td>Absent</td><td>n/a</td></tr>
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<tr><td>14:00</td><td>110</td><td>78</td><td>0</td><td>Absent</td><td>n/a</td></tr>
</table>

Rabbit 29

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	200
Acoustic power (W)	46
Sonication time (s)	20
Energy (J)	920
Cooling time (s)	60
Number of sonications	9
Step between sonications (mm)	4
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 9 (direction from rabbit feet to tail) at step movement of 4 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm.

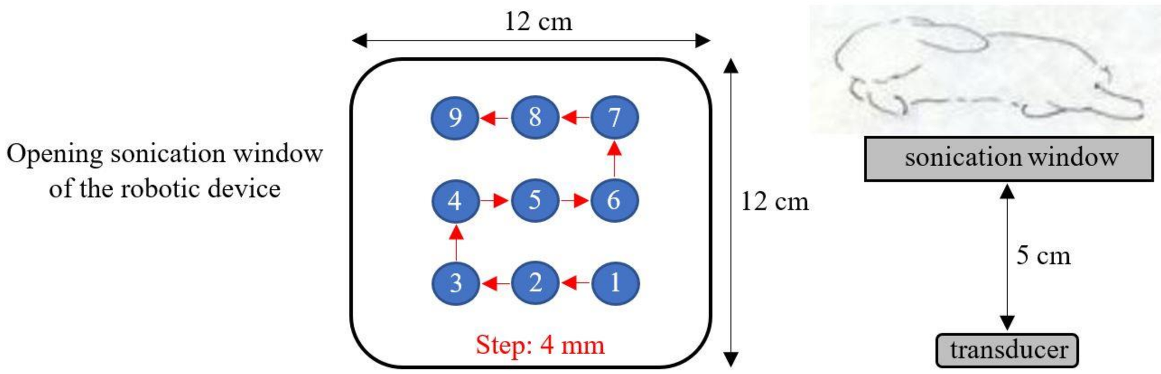

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	200
Acoustic power (W)	46
Sonication time (s)	10
Energy (J)	460
Cooling time (s)	60
Number of sonications	9
Step between sonications (mm)	4
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved from point 1 to 9 (direction from rabbit tail to feet) at step movement of 4 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm.

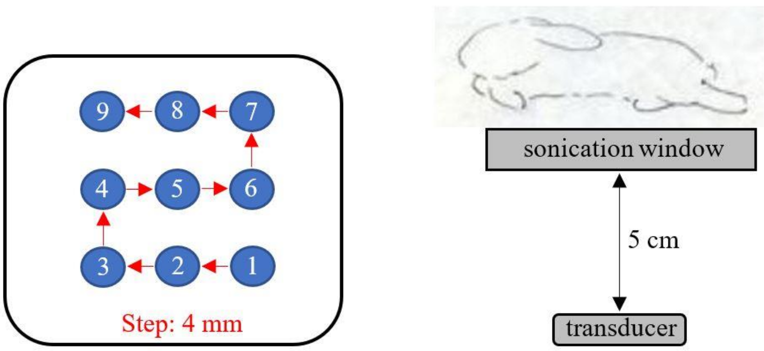

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory, that was used for the rabbit thigh ablations.

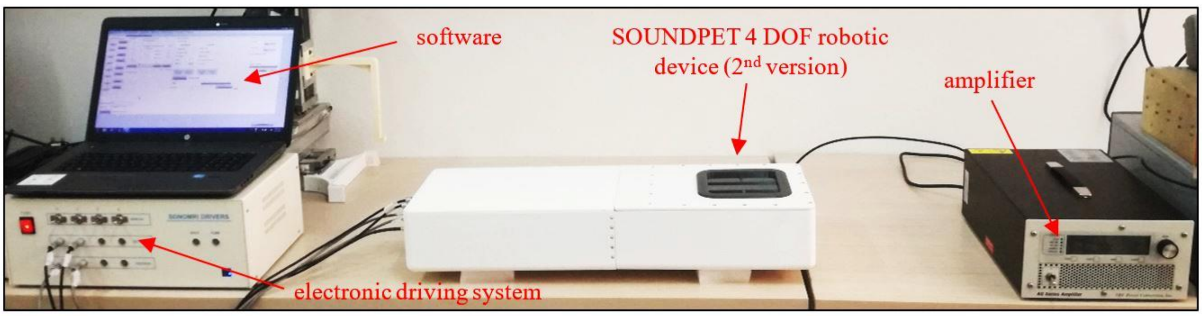

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

Figure 4: Animal positioning for the experiment.

Results

A necrotic area was appeared on the outer side (sonication side) of the left thigh above and below the skin as shown in Figure 5 and Figure 6, respectively.

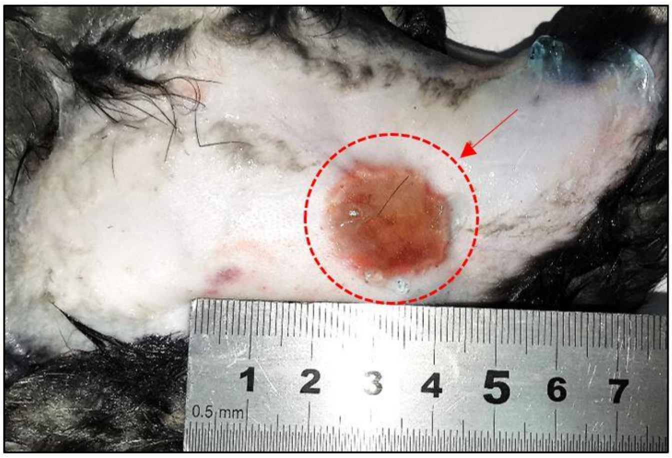

Figure 5: The outer side of the left thigh above the skin after the treatment. The red arrow indicates the area of the overlapping lesions (electric power 200 W for 20 s).

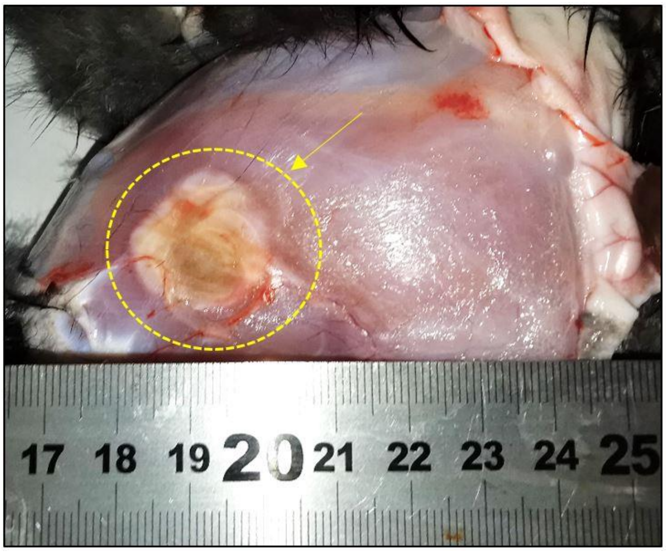

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side). The yellow arrow indicates the necrotic region (electric power 200 W for 20 s).

No lesions were appeared on the inner side of the left thigh above the skin as shown in Figure 7. However, a necrotic area was observed below the skin as shown in Figure 8.

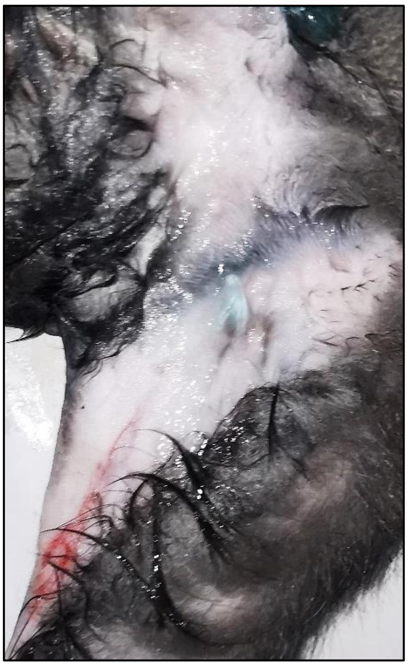

Figure 7: Macroscopic appearance of the inner side of the left thigh and above the skin. No lesions were observed (electric power 200 W for 20 s).

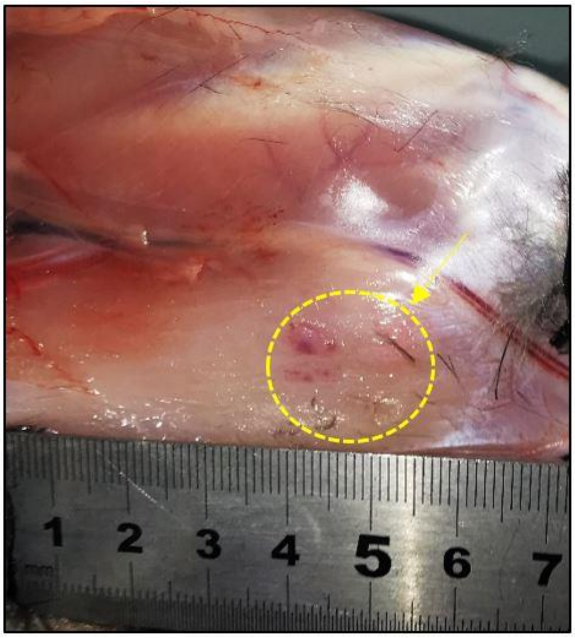

Figure 8: Macroscopic appearance of the exposed muscle from the inner side of the left thigh. A necrotic area (indicated with yellow arrow) was observed (electric power 200 W for 20 s).

The muscle of the left thigh was dissected and a necrotic region was observed as shown in Figure 9.

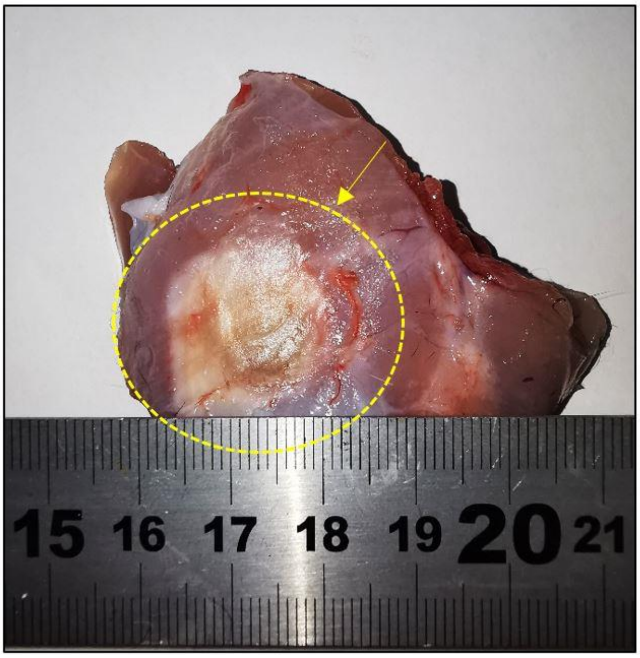

Figure 9: Macroscopic appearance of the overlapping lesions (indicated with yellow arrow, electric power 200 W for 20 s) on the dissected muscle of the outer side of the left thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 9 overlapping lesions on the surface of the muscle was calculated to be 19 mm x 19 mm while its depth was 12 mm. Figure 10 demonstrates the vertical cross middle dissection (with its mirror view) of the necrotic area that was created on the left thigh by applying a 3 x 3 grid pattern.

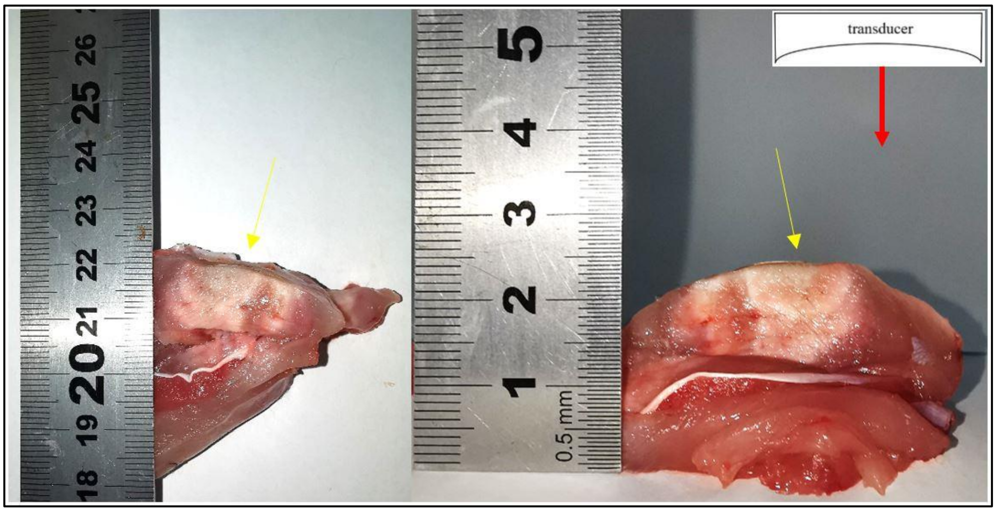

Figure 10: In situ dissection of the overlapping lesions that were created on the left thigh (electric power 200 W for 20 s). The yellow arrows indicate the necrotic area and the red arrow indicates the beam direction.

A necrotic area was also appeared on the outer side (sonication side) of the right thigh above and below the skin as shown in Figure 11 and Figure 12, respectively.

Figure 11: The outer side of the right thigh above the skin after the treatment (electric power 200 W for 10 s).

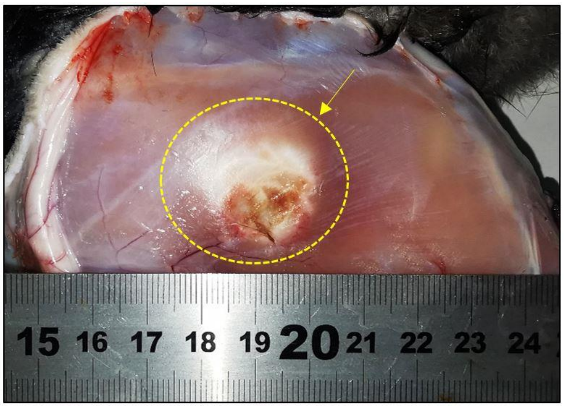

Figure 12: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed (electric power 200 W for 10 s).

No lesions were appeared on the inner side of the right thigh above and below the skin as shown in Figure 13 and Figure 14, respectively.

Figure 13: The inner side of the right thigh above the skin. No lesions were observed (electric power 200 W for 10 s).

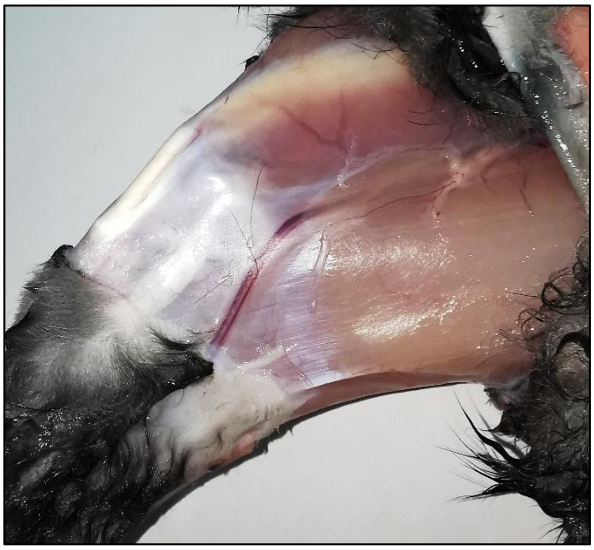

Figure 14: The exposed muscle from the inner side of the right thigh. No lesions were observed (electric power 200 W for 10 s).

The muscle of the right thigh was dissected and a necrotic region was observed as shown in Figure 15.

Figure 15: Macroscopic appearance of the overlapping lesions (indicated with yellow arrow, electric power 200 W for 10 s) on the dissected muscle of the outer side of the right thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 9 overlapping lesions on the surface of the muscle was calculated to be 19 mm x 20 mm while its depth was 19 mm. Figure 16 demonstrates the vertical cross middle dissection (with its mirror view) of the necrotic area that was created on the right thigh by applying a 3 x 3 grid pattern.

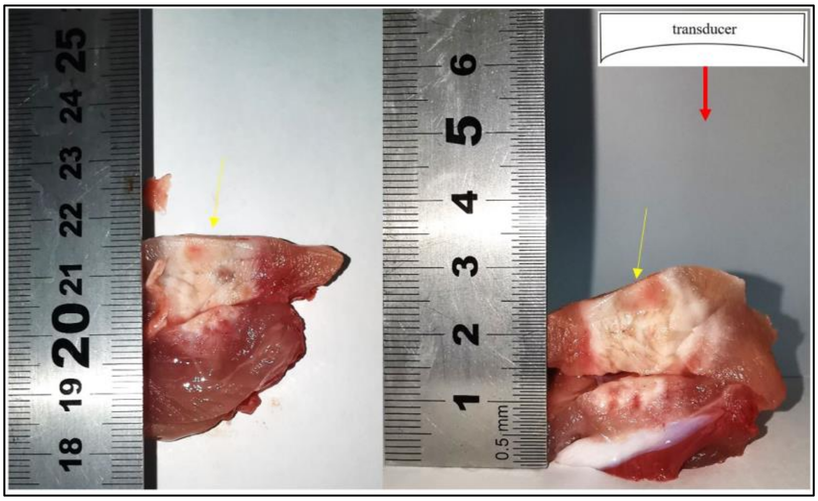

Figure 16: In situ dissection of the overlapping lesions that were created on the right thigh (electric power 200 W for 10 s). The yellow arrows indicate the necrotic area and the red arrow indicates the beam direction.

Table 3 lists the anesthesia record for the rabbit experiment.

Table 3: Anesthesia record for rabbit 29.

<table>
<tr><td colspan="3">Animal ID</td><td colspan="3">SOUNDPET_2020_29</td></tr>
<tr><td colspan="3">Species</td><td colspan="3">Rabbit</td></tr>
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<tr><td colspan="3">Age</td><td colspan="3">Adult</td></tr>
<tr><td colspan="3">Weight (Kg)</td><td colspan="3">2.95</td></tr>
<tr><td colspan="3">Supplier</td><td colspan="3">S. Ioannou Farm (CYCU5.05)</td></tr>
<tr><td colspan="3">Grant</td><td colspan="3">SOUNDPET (INTEGRATED/0918/0008)</td></tr>
<tr><td colspan="3">Study</td><td colspan="3">CY/EXP/PR.L01/2020</td></tr>
<tr><td colspan="3" rowspan="3">Participants</td><td colspan="3">Kyriakos Spanoudes</td></tr>
<tr><td colspan="3">Theocharis Drakos</td></tr>
<tr><td colspan="3">Nikolas Evripidou</td></tr>
<tr><td colspan="3">Principal Investigator</td><td colspan="3">Christakis Damianou</td></tr>
<tr><td colspan="6"></td></tr>
<tr><td colspan="3">Time</td><td colspan="3">Anesthesia</td></tr>
<tr><td colspan="3">09:30</td><td colspan="3">Medetomidine 1.5 ml + Ketamine 0.45 ml</td></tr>
<tr><td colspan="3">09:35</td><td colspan="3">Midazolam 0.5 ml</td></tr>
<tr><td colspan="3">Euthanasia</td><td colspan="3">10:15 1 ml T-61</td></tr>
<tr><td colspan="6"></td></tr>
<tr><td>Time</td><td>Heart Rate</td><td>Resp. Rate</td><td>Urination (0 to 3)</td><td>Absence of Movement /Pedal Reflex</td><td>Temp. (°C)</td></tr>
<tr><td>09:40</td><td>100</td><td>60</td><td>1</td><td>Absent</td><td>n/a</td></tr>
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<tr><td>10:10</td><td>100</td><td>64</td><td>0</td><td>Absent</td><td>n/a</td></tr>
</table>

Rabbit 30

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	200
Acoustic power (W)	46
Sonication time (s)	5
Energy (J)	230
Cooling time (s)	60
Number of sonications	9
Step between sonications (mm)	4
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 9 (direction from rabbit feet to tail) at step movement of 4 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm.

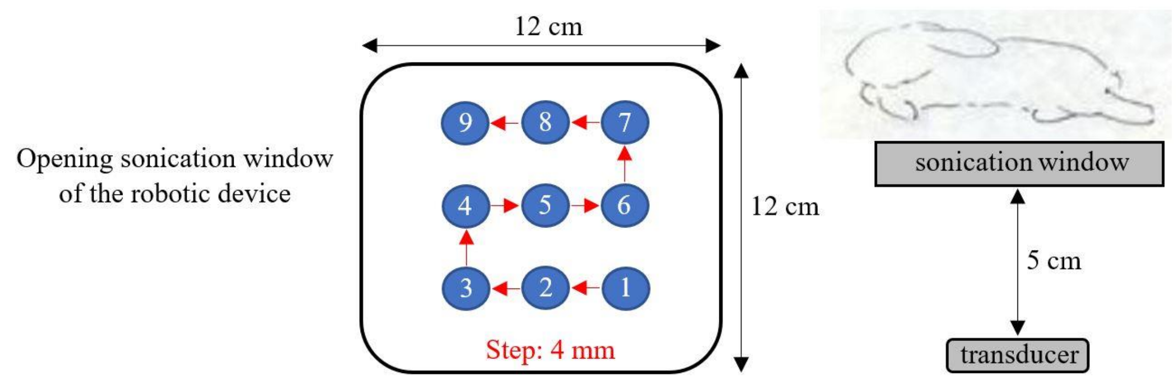

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	200
Acoustic power (W)	46
Sonication time (s)	10
Energy (J)	460
Cooling time (s)	60
Number of sonications	9
Step between sonications (mm)	4
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved from point 1 to 9 (direction from rabbit tail to feet) at step movement of 4 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm.

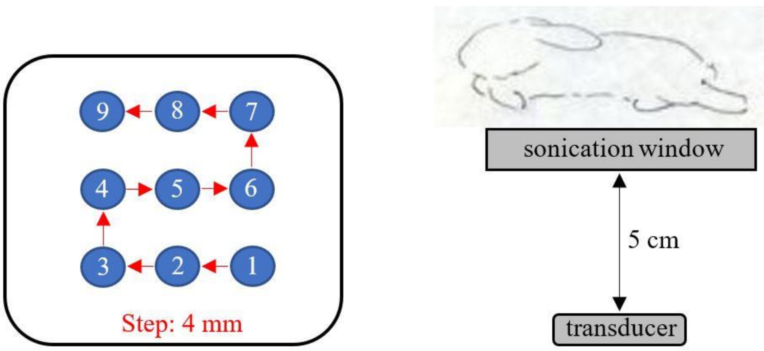

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory, that was used for the rabbit thigh ablations.

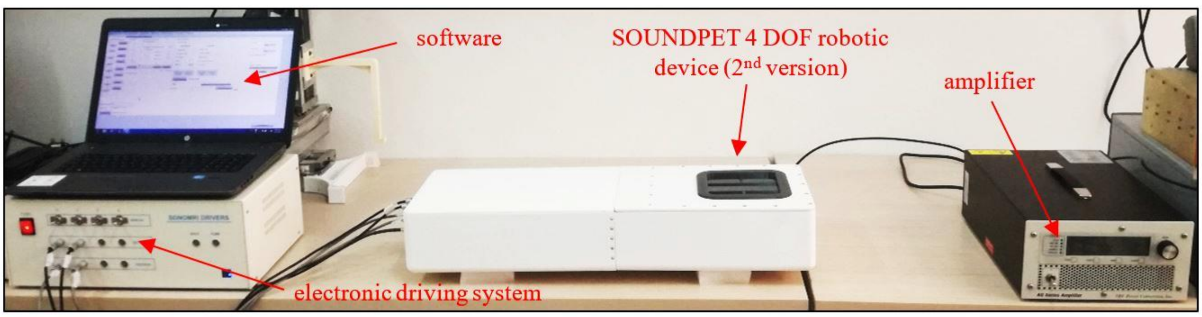

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

Figure 4: Animal positioning for the experiment.

Results

No lesions were appeared on the outer side (sonication side) of the left thigh above the skin as shown in Figure 5 while a necrotic area was observed below the skin as shown in Figure 6.

Figure 5: The outer side of the left thigh above the skin after the treatment. No lesions were observed (electric power 200 W for 5 s).

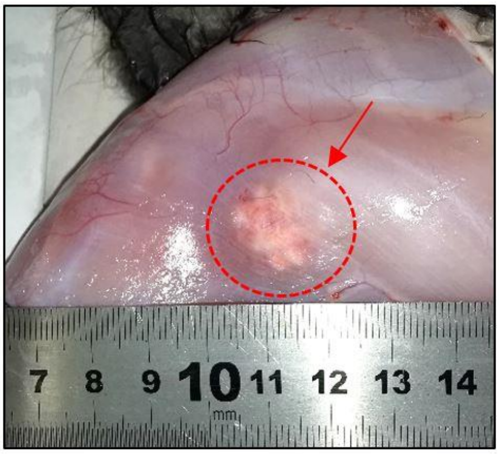

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side). The red arrow indicates the necrotic region (electric power 200 W for 5 s).

No lesions were appeared on the inner side of the left thigh above and below the skin as shown in Figure 7 and Figure 8, respectively.

Figure 7: Macroscopic appearance of the inner side of the left thigh and above the skin. No lesions were observed (electric power 200 W for 5 s).

Figure 8: Macroscopic appearance of the exposed muscle from the inner side of the left thigh. No lesions were observed (electric power 200 W for 5 s).

The muscle of the left thigh was dissected and a necrotic region was observed as shown in Figure 9.

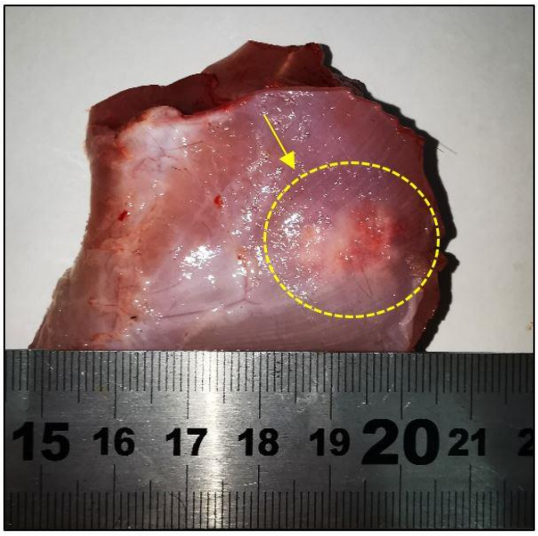

Figure 9: Macroscopic appearance of the overlapping lesions (indicated with yellow arrow, electric power 200 W for 5 s) on the dissected muscle of the outer side of the left thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 9 overlapping lesions on the surface of the muscle was calculated to be 14 mm x 10 mm while its depth was 15 mm. Figure 10 demonstrates the vertical cross middle dissection (with its mirror view) of the necrotic area that was created on the left thigh by applying a 3 x 3 grid pattern.

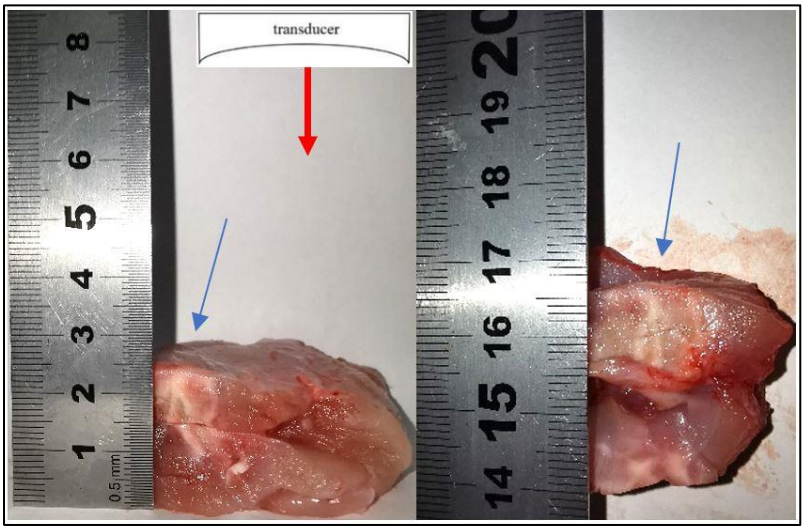

Figure 10: In situ dissection of the overlapping lesions that were created on the left thigh (electric power 200 W for 5 s). The blue arrows indicate the necrotic area and the red arrow indicates the beam direction.

A necrotic area was also appeared on the outer side (sonication side) of the right thigh above and below the skin as shown in Figure 11 and Figure 12, respectively.

Figure 11: The outer side of the right thigh above the skin after the treatment (electric power 200 W for 10 s). The red arrow indicates the necrotic region.

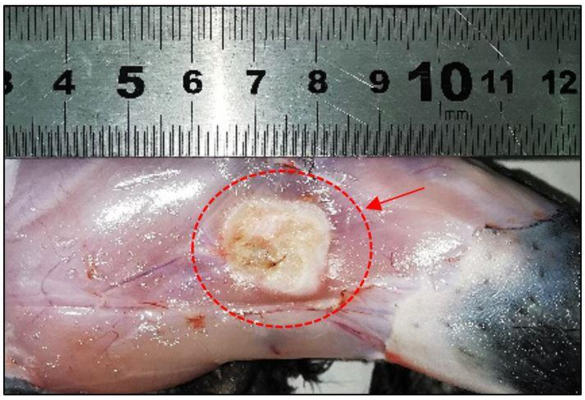

Figure 12: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed (electric power 200 W for 10 s). The red arrow indicates the necrotic region.

No lesions were appeared on the inner side of the right thigh above and below the skin as shown in Figure 13 and Figure 14, respectively.

Figure 13: The inner side of the right thigh above the skin. No lesions were observed (electric power 200 W for 10 s).

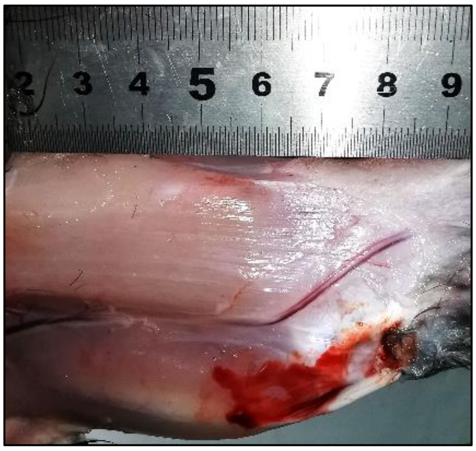

Figure 14: The exposed muscle from the inner side of the right thigh. No lesions were observed (electric power 200 W for 10 s).

The muscle of the right thigh was dissected and a necrotic region was observed as shown in Figure 15.

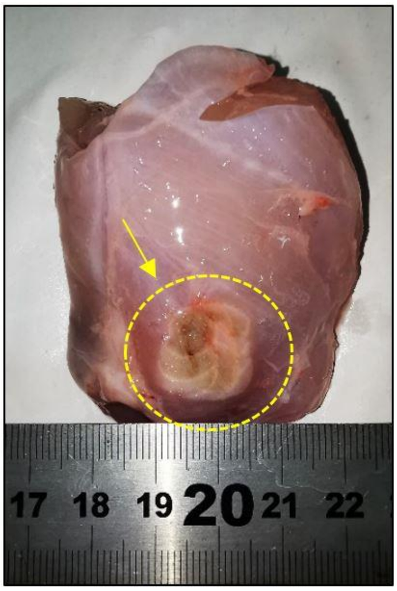

Figure 15: Macroscopic appearance of the overlapping lesions (indicated with yellow arrow, electric power 200 W for 10 s) on the dissected muscle of the outer side of the right thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 9 overlapping lesions on the surface of the muscle was calculated to be 15 mm x 15 mm while its depth was 11 mm. Figure 16 demonstrates the vertical cross middle dissection (with its mirror view) of the necrotic area that was created on the right thigh by applying a 3 x 3 grid pattern.

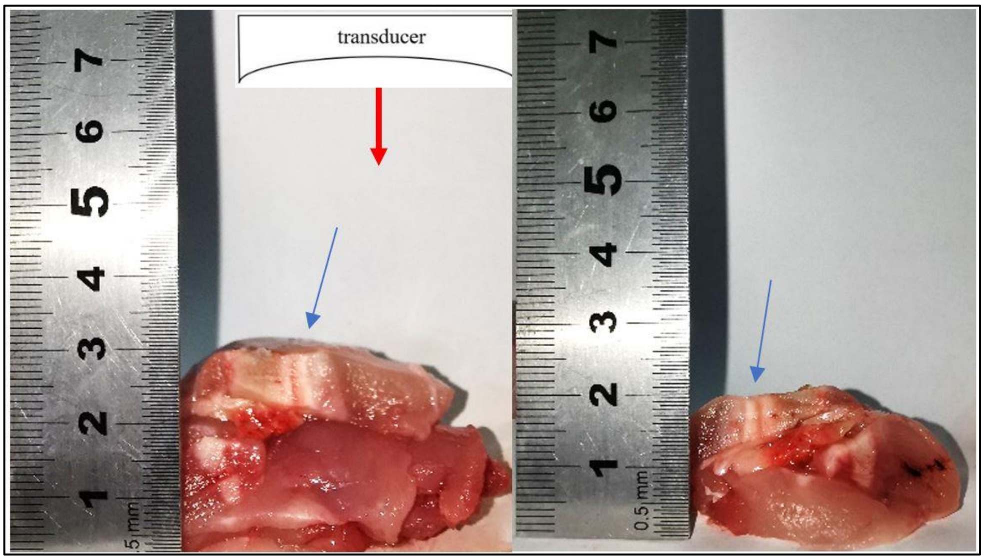

Figure 16: In situ dissection of the overlapping lesions that were created on the right thigh (electric power 200 W for 10 s). The blue arrows indicate the necrotic area and the red arrow indicates the beam direction.

Table 3 lists the anesthesia record for the rabbit experiment.

Table 3: Anesthesia record for rabbit 30.

Animal ID	SOUNDPET_2020_30				
Species	Rabbit				
Sex	F				
Age	Adult				
Weight (Kg)	3.05				
Supplier	S. Ioannou Farm (CYCU5.05)				
Grant	SOUNDPET (INTEGRATED/0918/0008)				
Study	CY/EXP/PR.L01/2020				
Participants	Kyriakos Spanoudes				
	Theocharis Drakos				
	Nikolas Evripidou				
Principal Investigator	Christakis Damianou				
Time	**Anesthesia**				
10:30	Medetomidine 1.5 ml + Ketamine 0.45 ml				
10:35	Midazolam 0.5 ml				
Euthanasia	11:15 1 ml T-61				
Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
10:40	110	62	0	Absent	n/a
10:50	110	68	0	Absent	n/a
11:00	100	62	0	Absent	n/a
11:10	100	66	0	Absent	n/a

Rabbit 31

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	250
Acoustic power (W)	57.5
Sonication time (s)	10
Energy (J)	575
Cooling time (s)	60
Number of sonications	16
Step between sonications (mm)	4
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 16 (direction from rabbit feet to tail) at step movement of 4 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm.

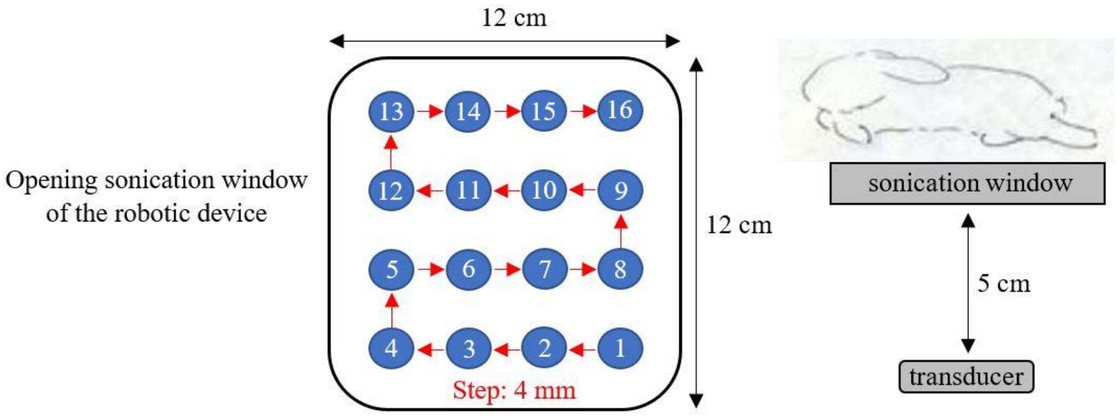

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	250
Acoustic power (W)	57.5
Sonication time (s)	5
Energy (J)	287.5
Cooling time (s)	60
Number of sonications	16
Step between sonications (mm)	4
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved from point 1 to 16 (direction from rabbit tail to feet) at step movement of 4 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm.

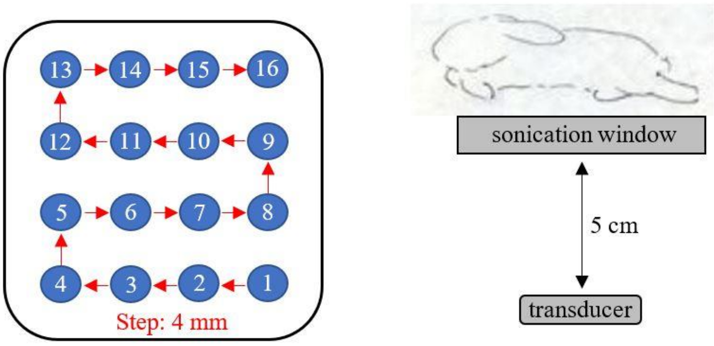

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory, that was used for the rabbit thigh ablations.

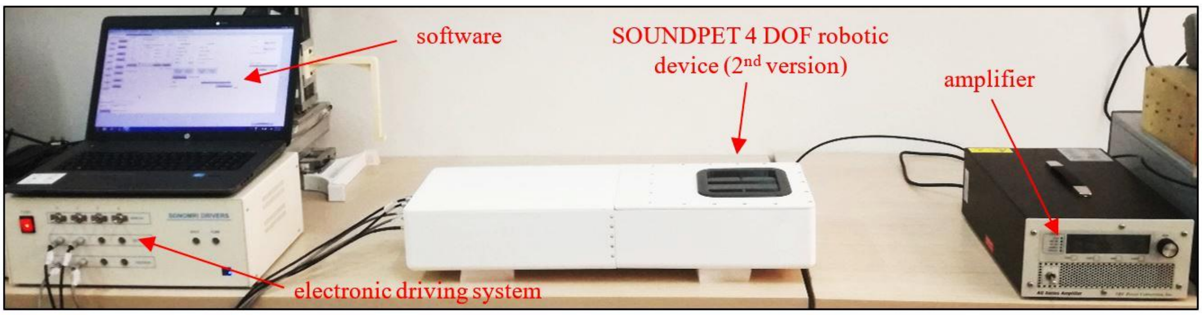

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

Figure 4: Animal positioning for the experiment.

Results

A necrotic area was appeared on the outer side (sonication side) of the left thigh above and below the skin as shown in Figure 5 and Figure 6, respectively. During the experiment, the rabbit slightly moved with a possible reason of sonicating the sciatic nerve which causes movement without any rabbit pain. This caused overlapping lesions at two locations on the muscle.

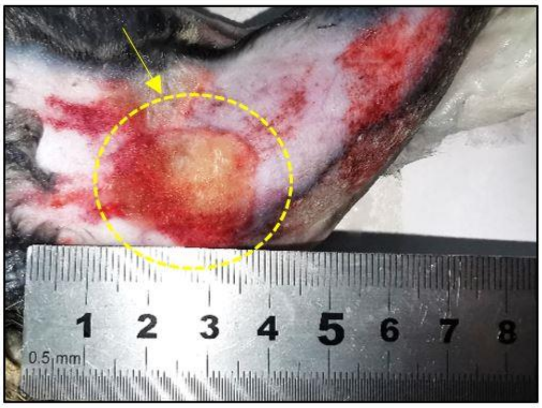

Figure 5: The outer side of the left thigh above the skin after the treatment. The yellow arrow indicates the necrotic area (electric power 250 W for 10 s).

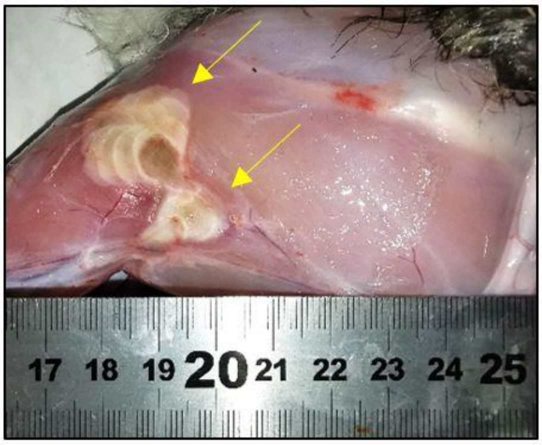

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side). The yellow arrows indicate the two necrotic areas which slightly overlapped (electric power 250 W for 10 s).

Some lesions were appeared on the inner side of the left thigh above and below the skin as shown in Figure 7 and Figure 8, respectively.

Figure 7: Macroscopic appearance of some lesions which were observed on the inner side of the left thigh and above the skin. The yellow arrow indicates the lesions on the skin (electric power 250 W for 10 s).

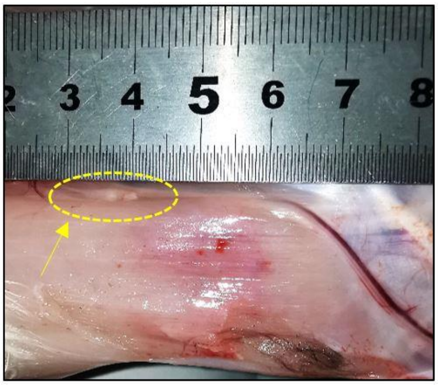

Figure 8: Macroscopic appearance of the exposed muscle from the inner side of the left thigh. Some lesions were appeared on the inner side after performing a 4 x 4 grid at step movement of 4 mm. The yellow arrow indicates the lesions on the exposed muscle (electric power 250 W for 10 s).

The muscle of the left thigh was dissected and a necrotic region was observed as shown in Figure 9.

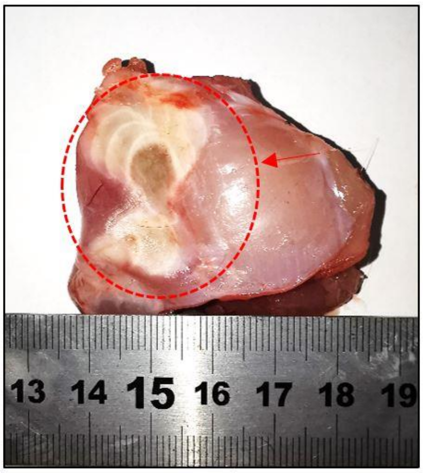

Figure 9: Macroscopic appearance of the overlapping lesions (indicated with red arrow, electric power 250 W for 10 s) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The area of the upper necrotic region was calculated to be 20 mm x 14 mm while of the lower necrotic region was 14 mm x 11 mm. The depth of the overlapping lesions was 17 mm. Figure 10 demonstrates the vertical cross middle dissection of the necrotic area (with its mirror view) that was created on the left thigh by applying a 4 x 4 grid pattern.

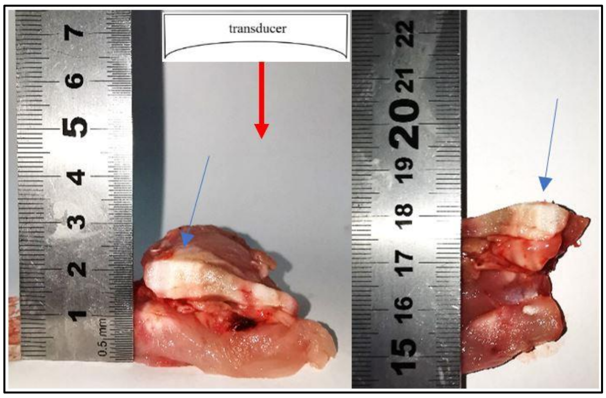

Figure 10: In situ dissection of the overlapping lesions that were created on the left thigh (electric power 250 W for 10 s). The blue arrows indicate the necrotic area and the red arrow indicates the beam direction.

As shown in Figure 11, no lesions were appeared on the outer side of the right thigh above the skin while a necrotic area was observed below the skin as shown in Figure 12.

Figure 11: The outer side of the right thigh above the skin after the treatment (electric power 250 W for 5 s).

Figure 12: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed. The red arrow indicates the necrotic region (electric power 250 W for 5 s).

Some lesions were appeared on the inner side of the right thigh above the skin as shown in Figure 13 while no lesions were observed below the skin as shown in Figure 14.

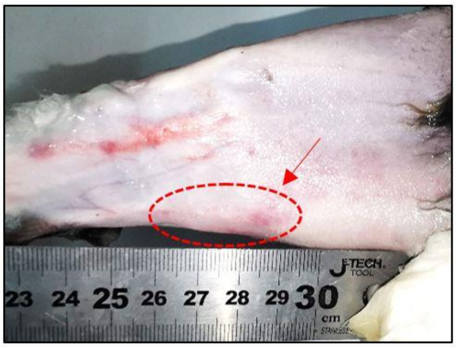

Figure 13: Macroscopic appearance of the lesions that were appeared on the inner side of the right thigh and above the skin. The red arrow indicates the area of the created overlapping lesions on the skin (electric power 250 W for 5 s).

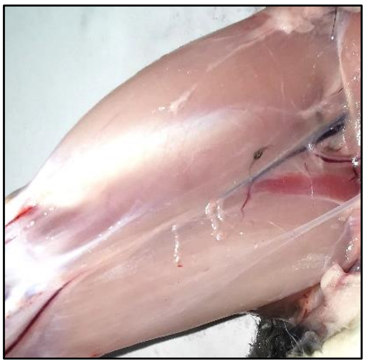

Figure 14: Macroscopic appearance of exposed muscle from the inner side of the right thigh. No lesions were observed (electric power 250 W for 5 s).

The muscle of the right thigh was dissected and a necrotic region was observed as shown in Figure 15. The location of the sonications was close to the bone and most of the sonications hit the bone. However, some sonications hit the muscle and necrosis was created.

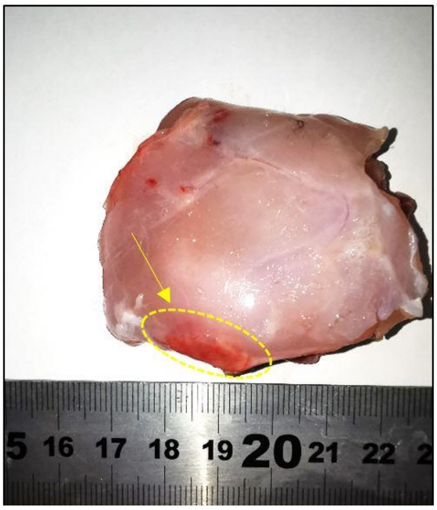

Figure 15: Macroscopic appearance of the overlapping lesions (indicated with yellow arrow, electric power 250 W for 5 s) on the dissected muscle of the outer side of the right thigh (plane perpendicular to the beam).

The area of the necrotic region that was observed on the surface of the muscle was calculated to be 16 mm x 6 mm while its depth was 4 mm. Figure 16 demonstrates the depth of the necrotic area that was created on the right thigh by applying a 4 x 4 grid pattern.

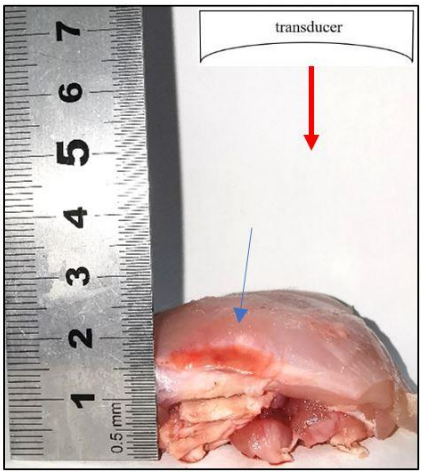

Figure 16: In situ dissection of the overlapping lesions that were created on the right thigh (electric power 250 W for 5 s). The blue arrows indicate the necrotic area and the red arrow indicates the beam direction.

Table 3 lists the anesthesia record for the rabbit experiment.

Table 3: Anesthesia record for rabbit 31.

<table>
<tr><td colspan="3">Animal ID</td><td colspan="3">SOUNDPET_2020_31</td></tr>
<tr><td colspan="3">Species</td><td colspan="3">Rabbit</td></tr>
<tr><td colspan="3">Sex</td><td colspan="3">F</td></tr>
<tr><td colspan="3">Age</td><td colspan="3">Adult</td></tr>
<tr><td colspan="3">Weight (Kg)</td><td colspan="3">3.15</td></tr>
<tr><td colspan="3">Supplier</td><td colspan="3">S. Ioannou Farm (CYCU5.05)</td></tr>
<tr><td colspan="3">Grant</td><td colspan="3">SOUNDPET (INTEGRATED/0918/0008)</td></tr>
<tr><td colspan="3">Study</td><td colspan="3">CY/EXP/PR.L01/2020</td></tr>
<tr><td colspan="3" rowspan="3">Participants</td><td colspan="3">Kyriakos Spanoudes</td></tr>
<tr><td colspan="3">Theocharis Drakos</td></tr>
<tr><td colspan="3">Nikolas Evripidou</td></tr>
<tr><td colspan="3">Principal Investigator</td><td colspan="3">Christakis Damianou</td></tr>
<tr><td colspan="6"></td></tr>
<tr><td colspan="3">Time</td><td colspan="3">Anesthesia</td></tr>
<tr><td colspan="3">12:50</td><td colspan="3">Medetomidine 1.5 ml + Ketamine 0.5 ml</td></tr>
<tr><td colspan="3">13:05</td><td colspan="3">Midazolam 0.5 ml</td></tr>
<tr><td colspan="3">Euthanasia</td><td colspan="3">13:55 1 ml T-61</td></tr>
<tr><td colspan="6"></td></tr>
<tr><td>Time</td><td>Heart Rate</td><td>Resp. Rate</td><td>Urination
(0 to 3)</td><td>Absence of Movement /Pedal Reflex</td><td>Temp. (°C)</td></tr>
</table>

13:10	110	62	0	Absent	n/a
13:20	110	66	0	Absent	n/a
13:30	100	66	0	Absent	n/a
13:40	110	64	0	Absent	n/a
13:50	100	62	1	Absent	n/a

Rabbit 32

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	250
Acoustic power (W)	57.5
Sonication time (s)	5
Energy (J)	287.5
Cooling time (s)	60
Number of sonications	16
Step between sonications (mm)	4
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 16 (direction from rabbit feet to tail) at step movement of 4 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm. In this experiment, we did not include a water-filled glove and only ultrasound gel was added between the rabbit's legs. The purpose of not including a water-filled glove was to check the possibility of the ultrasound beam to reach the other inner side of the other leg (which it did not sonicated) and create lesion.

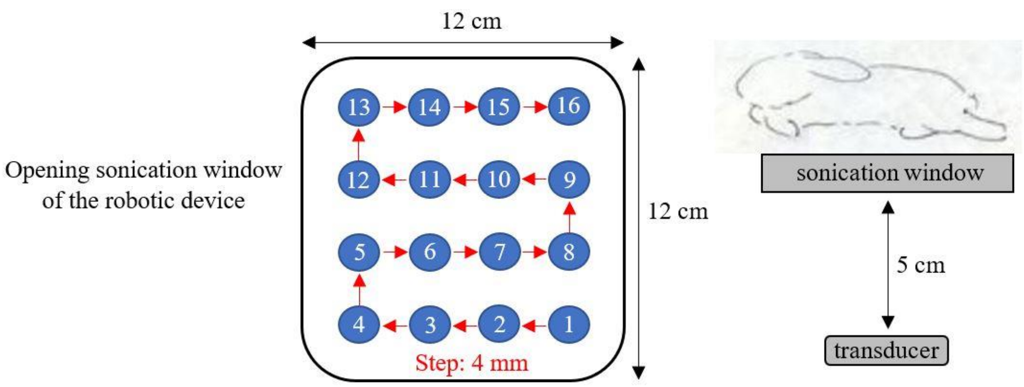

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Table 2 lists the settings that were used for the sonications on the right thigh.

Table 2: Settings for right thigh.

Parameter	Value
Electric power (W)	250
Acoustic power (W)	57.5
Sonication time (s)	3
Energy (J)	172.5
Cooling time (s)	60
Number of sonications	16
Step between sonications (mm)	4
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the right thigh ablations as shown in Figure 2. The robotic device was moved from point 1 to 16 (direction from rabbit tail to feet) at step movement of 4 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm.

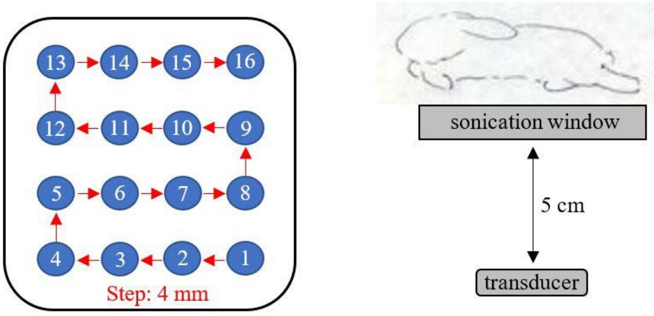

Figure 2: Grid movement for ablations on the right thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 3 shows the experimental set-up in the laboratory, that was used for the rabbit thigh ablations.

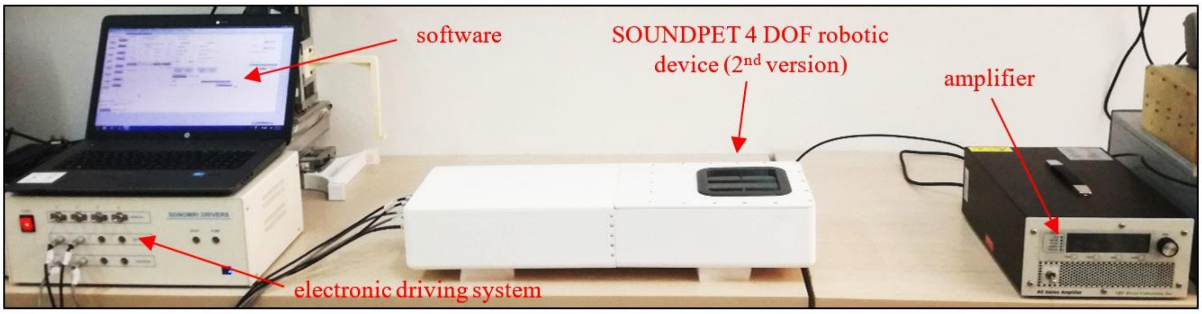

Figure 3: Experimental set-up for rabbit thigh ablation.

Figure 4 shows the anesthetized rabbit positioning for thigh ablation.

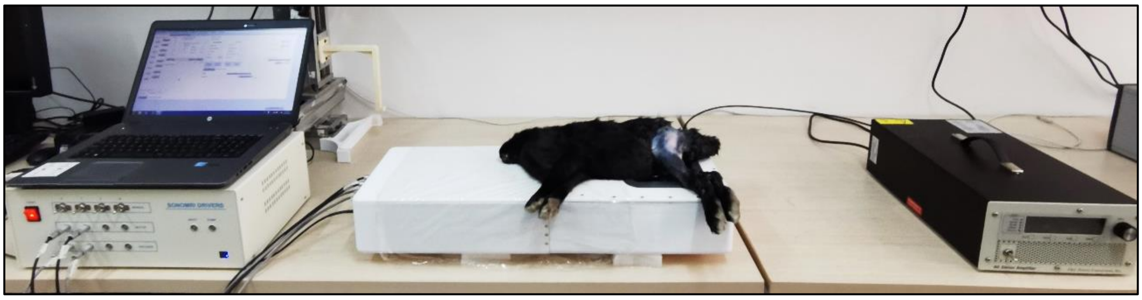

Figure 4: Animal positioning for the experiment.

Results

No lesions were appeared on the outer side (sonication side) of the left thigh above the skin as shown in Figure 5 while a necrotic area was observed below the skin as shown in Figure 6.

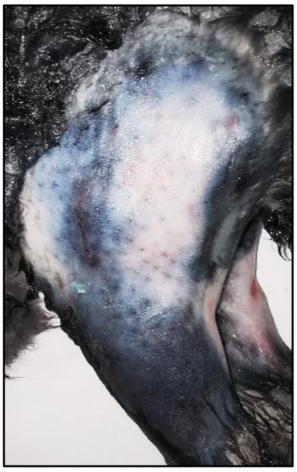

Figure 5: The outer side of the left thigh above the skin after the treatment. No lesions were observed (electric power 250 W for 5 s).

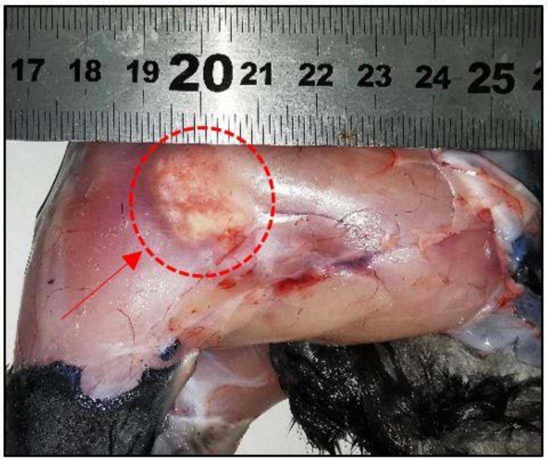

Figure 6: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side). The red arrows indicate the necrotic area (electric power 250 W for 5 s).

Overlapping lesions were appeared on the inner side of the left thigh above and below the skin as shown in Figure 7 and Figure 8, respectively.

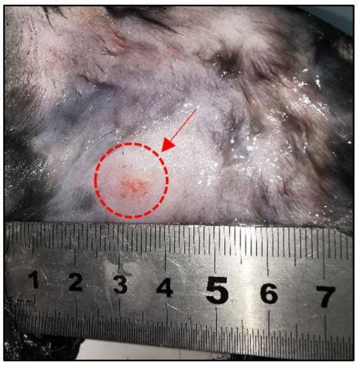

Figure 7: Macroscopic appearance of the overlapping lesions from the inner side of the left thigh and above the skin. The red arrow indicates the area of the created overlapping lesions on the skin (electric power 250 W for 5 s).

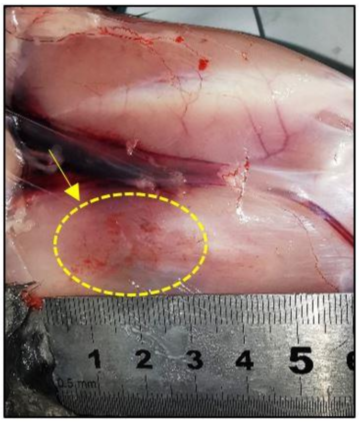

Figure 8: Macroscopic appearance of the exposed muscle from the inner side of the left thigh. Overlapping lesions were formed after performing a 4 x 4 grid at step movement of 4 mm. The yellow arrow indicates the area of the created overlapping lesions on the exposed muscle (electric power 250 W for 5 s).

The muscle of the left thigh was dissected and a necrotic region was observed as shown in Figure 9.

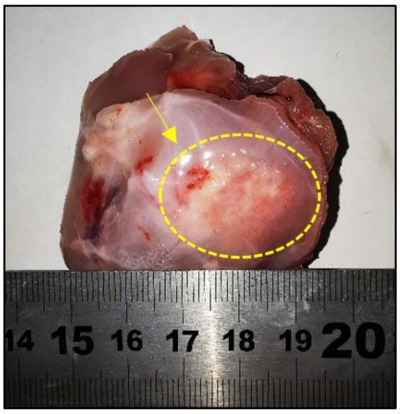

Figure 9: Macroscopic appearance of the overlapping lesions (indicated with red arrow, electric power 250 W for 5 s) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 16 overlapping lesions on the surface of the muscle was calculated to be 19 mm x 15 mm while its depth was 17 mm. Figure 10 demonstrates the vertical cross middle dissection of the necrotic area (with its mirror view) that was created on the left thigh by applying a 4 x 4 grid pattern.

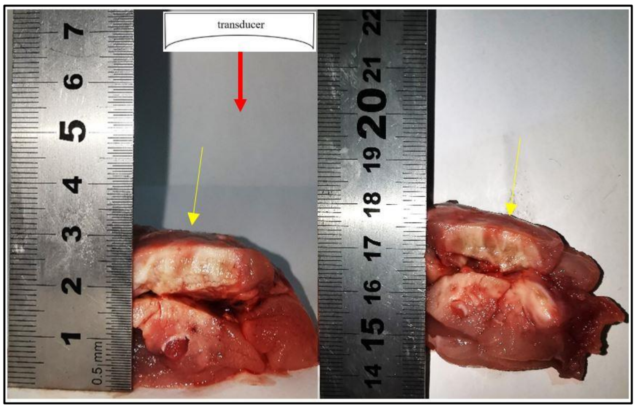

Figure 10: In situ dissection of the overlapping lesions that were created on the left thigh (electric power 250 W for 5 s). The yellow arrows indicate the necrotic area and the red arrow indicates the beam direction.

Overlapping lesions appeared above the skin as shown in Figure 11. However, no lesions were appeared on the outer side of the right thigh below the skin as shown in Figure 12.

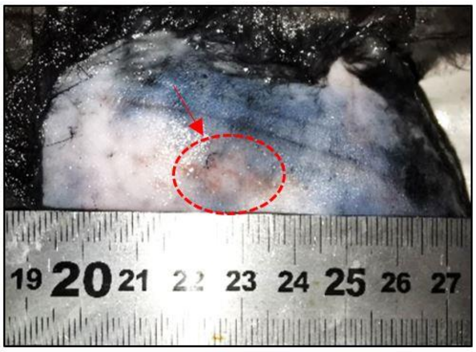

Figure 11: The outer side of the right thigh above the skin after the treatment. The red arrow indicates the area of the created overlapping lesions on the skin (electric power 250 W for 3 s).

Figure 12: The outer side of the right thigh following treatment and when the skin was removed and the muscle was exposed (electric power 250 W for 3 s).

No lesions were appeared on the inner side of the right thigh above the skin as shown in Figure 13. However, discrete lesions were appeared below the skin as shown in Figure 14.

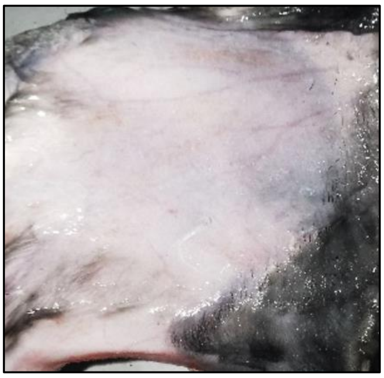

Figure 13: Macroscopic appearance of the inner side of the right thigh above the skin (electric power 250 W for 3 s).

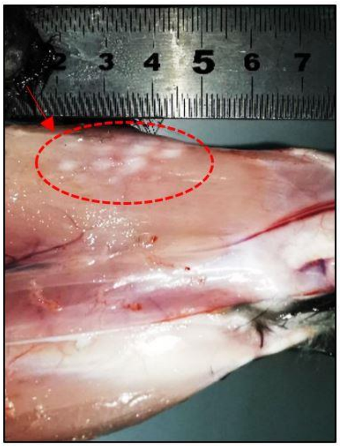

Figure 14: Macroscopic appearance of the discrete lesions on the exposed muscle from the inner side of the right thigh. The yellow arrows indicate the formed lesions after performing a 4 x 4 grid at step movement of 4 mm (electric power 250 W for 3 s).

The muscle of the right thigh was dissected and discrete lesions were observed on the surface of the muscle as shown in Figure 15.

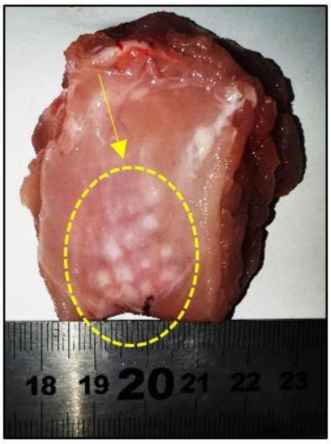

Figure 15: Macroscopic appearance of the discrete lesions (indicated with yellow arrow, electric power 250 W for 3 s) on the dissected muscle of the outer side of the right thigh (plane perpendicular to the beam).

The average diameter and length of the discrete lesions was 3 mm and 4 mm, respectively. Figure 16 demonstrates the vertical cross middle dissection of the discrete lesions that were created on the right thigh by applying a 4 x 4 grid pattern.

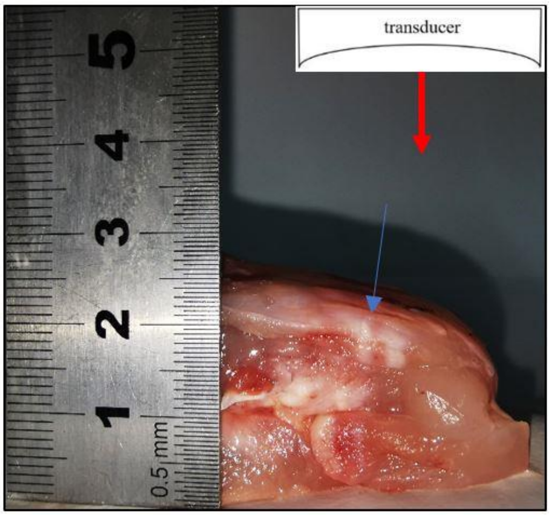

Figure 16: In situ dissection of the discrete lesions that were created on the right thigh (electric power 250 W for 3 s). The blue arrow indicates the lesions and the red arrow indicates the beam direction.

Table 3 lists the anesthesia record for the rabbit experiment.

Table 3: Anesthesia record for rabbit 32.

Animal ID			SOUNDPET_2020_32		
Species			Rabbit		
Sex			F		
Age			Adult		
Weight (Kg)			3.2		
Supplier			S. Ioannou Farm (CYCU5.05)		
Grant			SOUNDPET (INTEGRATED/0918/0008)		
Study			CY/EXP/PR.L01/2020		
Participants			Kyriakos Spanoudes		
			Theocharis Drakos		
			Nikolas Evripidou		
Principal Investigator			Christakis Damianou		
Time			**Anesthesia**		
14:30			Medetomidine 1.6 ml + Ketamine 0.5 ml		
14:40			Midazolam 0.5 ml		
Euthanasia			15:30 1 ml T-61		
Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement**	**Temp. (°C)**

				/Pedal Reflex	
14:45	100	72	0	Absent	n/a
15:00	110	72	0	Absent	n/a
15:10	110	68	0	Absent	n/a
15:20	100	68	2	Absent	n/a
15:30	110	64	0	Absent	n/a

Rabbit 33

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	250
Acoustic power (W)	57.5
Sonication time (s)	5
Energy (J)	287.5
Cooling time (s)	60
Number of sonications	9
Step between sonications (mm)	4
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 9 (direction from rabbit feet to tail) at step movement of 4 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm. In this experiment, we did not include a water-filled glove and only ultrasound gel was added between the rabbit's legs. The purpose of not including a water-filled glove was to check the possibility of the ultrasound beam to reach the other inner side of the other leg (which it did not sonicated) and create lesion. For that reason, only the left thigh of the rabbit was sonicated.

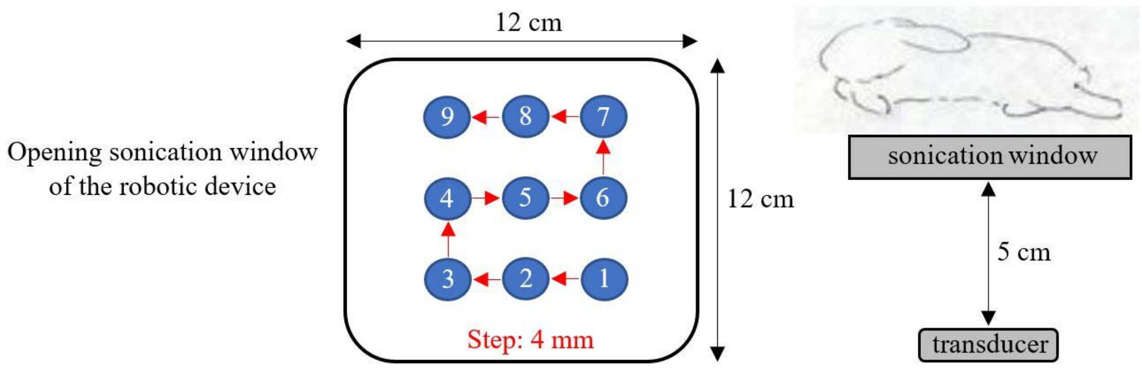

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 2 shows the experimental set-up in the laboratory, that was used for the rabbit thigh ablations.

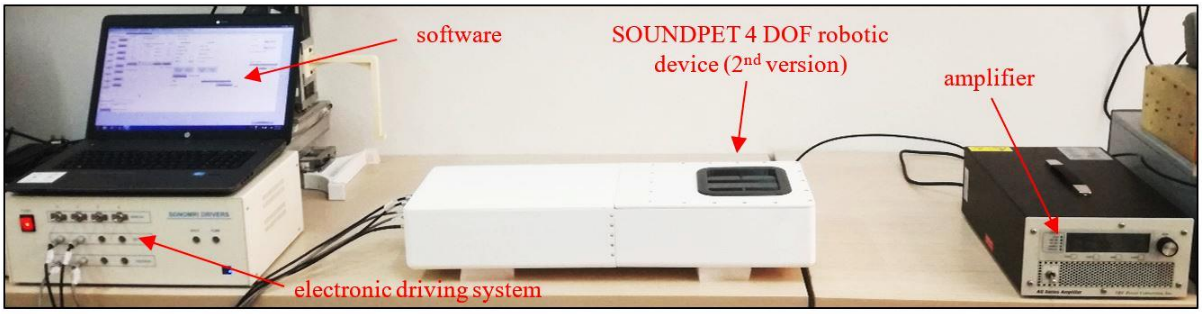

Figure 2: Experimental set-up for rabbit thigh ablation.

Figure 3 shows the anesthetized rabbit positioning for thigh ablation.

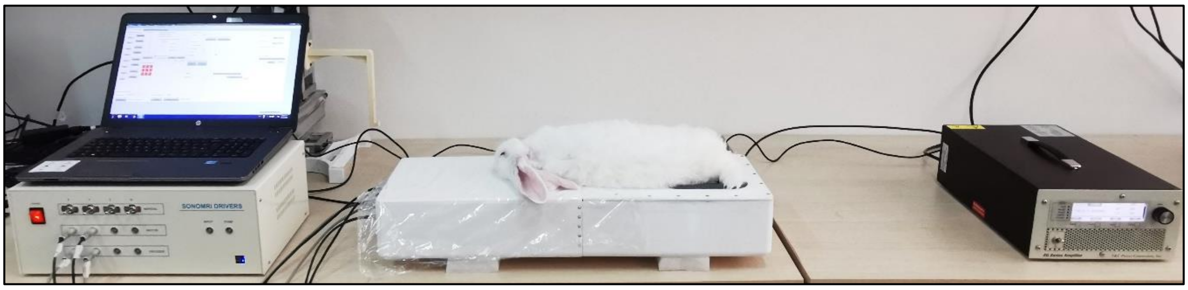

Figure 3: Animal positioning for the experiment.

Results

A necrotic area was appeared on the outer side (sonication side) of the left thigh above and below the skin as shown in Figure 4 and Figure 5, respectively.

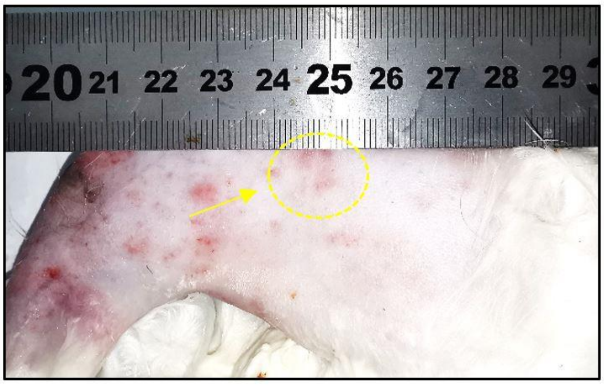

Figure 4: The outer side of the left thigh above the skin after the treatment. The yellow arrow indicates the necrotic area on the skin (electric power 250 W for 5 s).

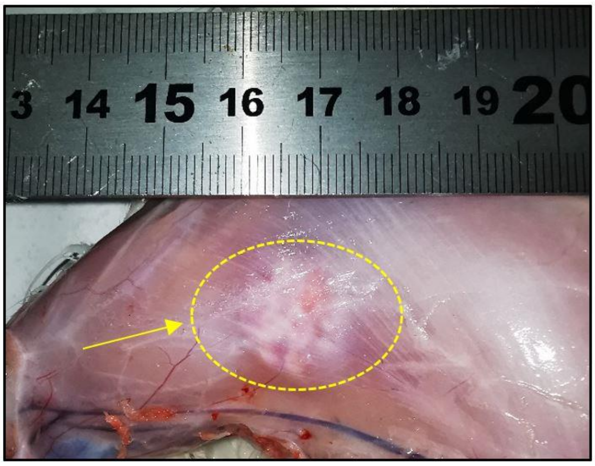

Figure 5: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side). The yellow arrow indicates the necrotic area (electric power 250 W for 5 s).

Overlapping lesions were appeared on the inner side of the left thigh above and below the skin as shown in Figure 6 and Figure 7, respectively.

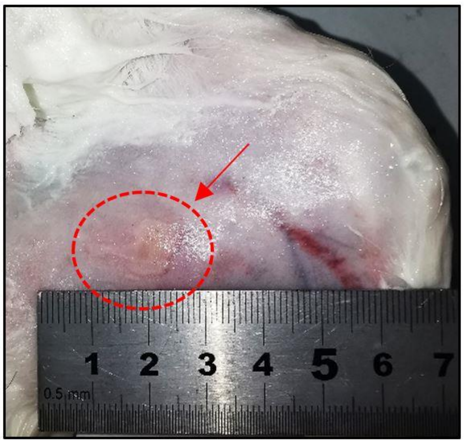

Figure 6: Macroscopic appearance of the overlapping lesions from the inner side of the left thigh and above the skin. The red arrow indicates the area of the created overlapping lesions on the skin (electric power 250 W for 5 s).

Figure 7: Macroscopic appearance of the exposed muscle from the inner side of the left thigh. Overlapping lesions were formed after performing a 3 x 3 grid at step movement of 4 mm. The red arrow indicates the area of the created overlapping lesions on the exposed muscle (electric power 250 W for 5 s).

One lesion was observed on the inner side of the right thigh above the skin as shown in Figure 8. The material between the two legs was ultrasound gel and only the left thigh was sonicated. No lesions were appeared below the skin of the inner side of the right thigh.

Figure 8: Macroscopic appearance of the inner side of the right thigh above the skin. The red arrow indicates the lesion on the skin (electric power 250 W for 5 s).

The muscle of the left thigh was dissected and a necrotic region was observed as shown in Figure 9.

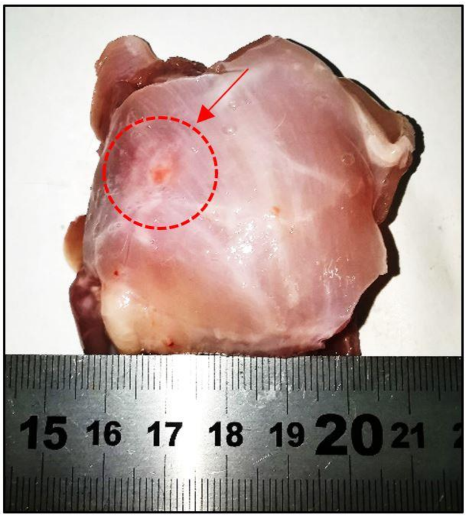

Figure 9: Macroscopic appearance of the overlapping lesions (indicated with red arrow, electric power 250 W for 5 s) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 9 overlapping lesions on the surface of the muscle was calculated to be 9 mm x 12 mm while its depth was 6 mm. Figure 10 demonstrates the vertical cross middle dissection of the necrotic area that was created on the left thigh by applying a 3 x 3 grid pattern.

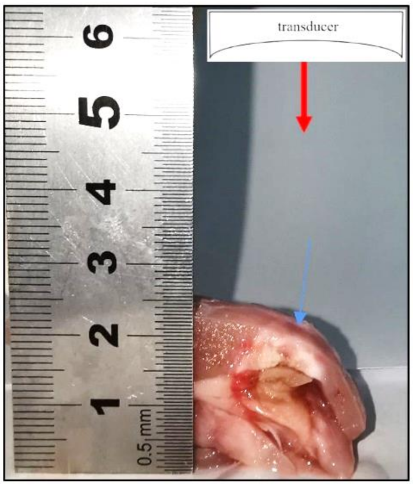

Figure 10: In situ dissection of the overlapping lesions that were created on the left thigh (electric power 250 W for 5 s). The blue arrow indicates the necrotic area and the red arrow indicates the beam direction.

Table 2 lists the anesthesia record for the rabbit experiment.

Table 2: Anesthesia record for rabbit 33.

<table>
<tr><th colspan="3">Animal ID</th><td colspan="3">SOUNDPET_2020_33</td></tr>
<tr><th colspan="3">Species</th><td colspan="3">Rabbit</td></tr>
<tr><th colspan="3">Sex</th><td colspan="3">F</td></tr>
<tr><th colspan="3">Age</th><td colspan="3">Adult</td></tr>
<tr><th colspan="3">Weight (Kg)</th><td colspan="3">2.49</td></tr>
<tr><th colspan="3">Supplier</th><td colspan="3">S. Ioannou Farm (CYCU5.05)</td></tr>
<tr><th colspan="3">Grant</th><td colspan="3">SOUNDPET (INTEGRATED/0918/0008)</td></tr>
<tr><th colspan="3">Study</th><td colspan="3">CY/EXP/PR.L01/2020</td></tr>
<tr><th colspan="3" rowspan="3">Participants</th><td colspan="3">Kyriakos Spanoudes</td></tr>
<tr><td colspan="3">Theocharis Drakos</td></tr>
<tr><td colspan="3">Nikolas Evripidou</td></tr>
<tr><th colspan="3">Principal Investigator</th><td colspan="3">Christakis Damianou</td></tr>
<tr><td colspan="6"></td></tr>
<tr><th colspan="3">Time</th><th colspan="3">Anesthesia</th></tr>
<tr><td colspan="3">09:40</td><td colspan="3">Medetomidine 1.5 ml + Ketamine 0.5 ml</td></tr>
<tr><td colspan="3">09:45</td><td colspan="3">Midazolam 0.5 ml</td></tr>
<tr><th colspan="3">Euthanasia</th><td colspan="3">10:15 1 ml T-61</td></tr>
<tr><td colspan="6"></td></tr>
<tr><th>Time</th><th>Heart Rate</th><th>Resp. Rate</th><th>Urination (0 to 3)</th><th>Absence of Movement /Pedal Reflex</th><th>Temp. (°C)</th></tr>
<tr><td>09:50</td><td>115</td><td>62</td><td>1</td><td>Absent</td><td>n/a</td></tr>
<tr><td>10:00</td><td>110</td><td>64</td><td>0</td><td>Absent</td><td>n/a</td></tr>
<tr><td>10:10</td><td>110</td><td>62</td><td>0</td><td>Absent</td><td>n/a</td></tr>
</table>

Rabbit 34

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	250
Acoustic power (W)	57.5
Sonication time (s)	3
Energy (J)	172.5
Cooling time (s)	60
Number of sonications	9
Step between sonications (mm)	3
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 9 (direction from rabbit feet to tail) at step movement of 3 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm. In this experiment, we did not include a water-filled glove and only ultrasound gel was added between the rabbit's legs. The purpose of not including a water-filled glove was to check the possibility of the ultrasound beam to reach the other inner side of the other leg (which it did not sonicated) and create lesion. For that reason, only the left thigh of the rabbit was sonicated.

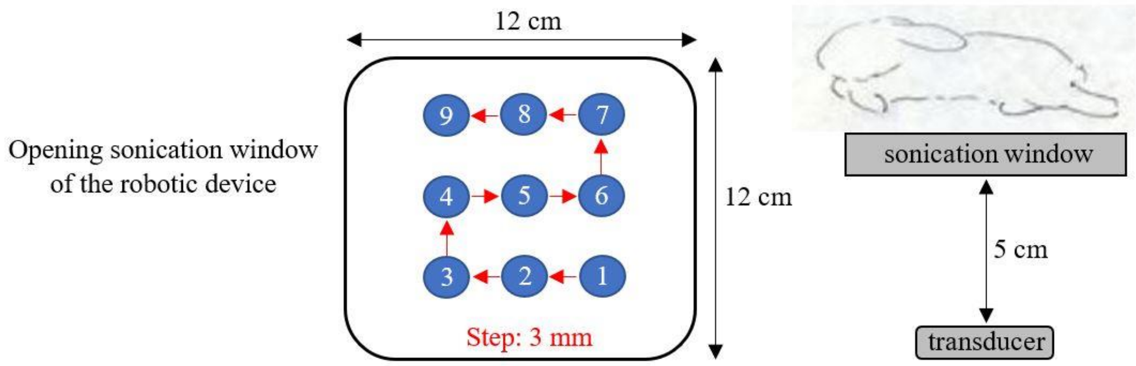

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 2 shows the experimental set-up in the laboratory, that was used for the rabbit thigh ablations.

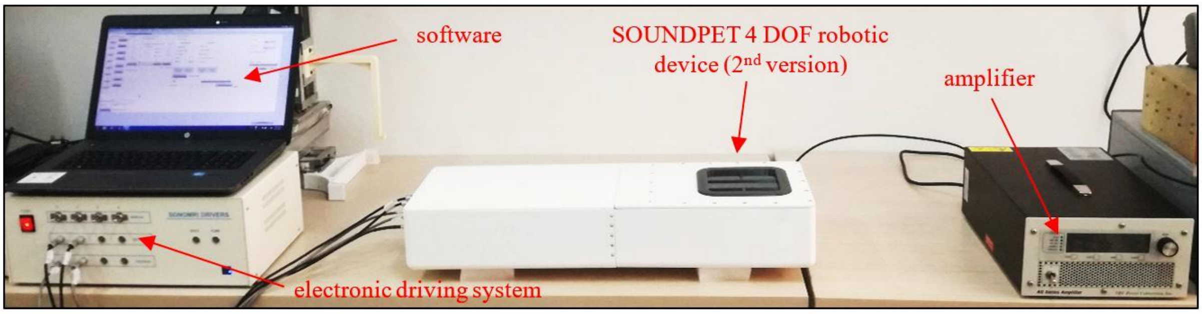

Figure 2: Experimental set-up for rabbit thigh ablation.

Figure 3 shows the anesthetized rabbit positioning for thigh ablation.

Figure 3: Animal positioning for the experiment.

Results

A necrotic area was appeared on the outer side (sonication side) of the left thigh above and below the skin as shown in Figure 4 and Figure 5, respectively.

Figure 4: The outer side of the left thigh above the skin after the treatment. The yellow arrow indicates the necrotic area on the skin (electric power 250 W for 3 s).

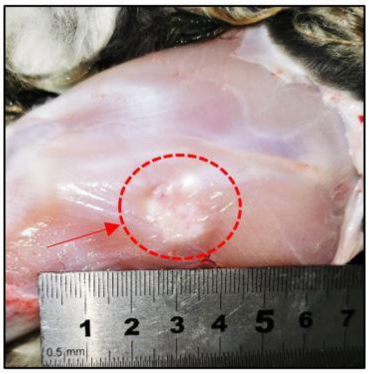

Figure 5: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side). The red arrow indicates the necrotic area (electric power 250 W for 3 s).

No lesions were observed on the inner side of the left thigh above the skin as shown in Figure 6 while two discrete lesions were observed below the skin as shown in Figure 7.

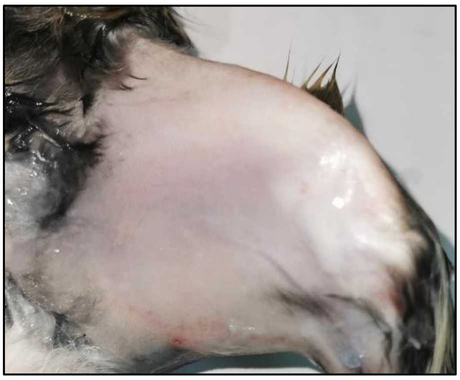

Figure 6: Macroscopic appearance of the inner side of the left thigh and above the skin. No lesions were observed on the skin (electric power 250 W for 3 s).

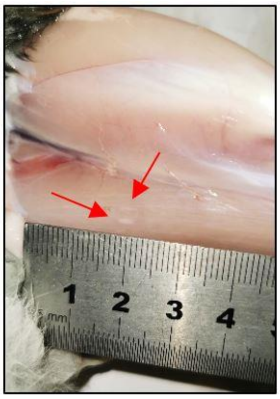

Figure 7: Macroscopic appearance of the exposed muscle from the inner side of the left thigh. The red arrows indicate the discrete lesions that were appeared on the exposed muscle (electric power 250 W for 3 s).

The muscle of the left thigh was dissected and a necrotic region was observed as shown in Figure 8.

Figure 8: Macroscopic appearance of the overlapping lesions (indicated with red arrow, electric power 250 W for 3 s) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 9 overlapping lesions on the surface of the muscle was calculated to be 7 mm x 14 mm while its depth was 14 mm. Figure 9 demonstrates the vertical cross middle dissection of the necrotic area (with its mirror view) that was created on the left thigh by applying a 3 x 3 grid pattern.

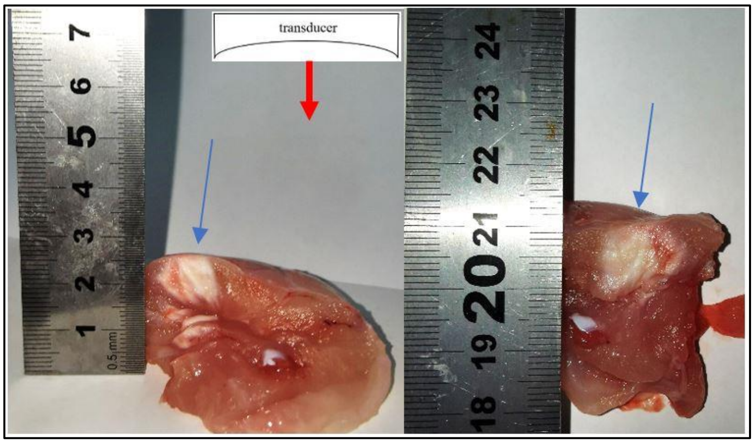

Figure 9: In situ dissection of the overlapping lesions that were created on the left thigh (electric power 250 W for 3 s). The blue arrows indicate the necrotic area and the red arrow indicates the beam direction.

Table 2 lists the anesthesia record for the rabbit experiment.

Table 2: Anesthesia record for rabbit 34.

Animal ID	SOUNDPET_2020_34
Species	Rabbit
Sex	M
Age	Adult
Weight (Kg)	2.6
Supplier	S. Ioannou Farm (CYCU5.05)
Grant	SOUNDPET (INTEGRATED/0918/0008)
Study	CY/EXP/PR.L01/2020

Participants	Kyriakos Spanoudes Theocharis Drakos Nikolas Evripidou
Principal Investigator	Christakis Damianou
Time	**Anesthesia**
10:10	Medetomidine 1.5 ml + Ketamine 0.5 ml
10:15	Midazolam 0.5 ml
Euthanasia	10:45 1 ml T-61

Time	Heart Rate	Resp. Rate	Urination (0 to 3)	Absence of Movement /Pedal Reflex	Temp. (°C)
10:15	100	66	0	Absent	n/a
10:30	100	66	0	Absent	n/a
10:40	110	68	0	Absent	n/a

Rabbit 35

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	250
Acoustic power (W)	57.5
Sonication time (s)	5
Energy (J)	287.5
Cooling time (s)	60
Number of sonications	9
Step between sonications (mm)	4
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 9 (direction from rabbit feet to tail) at step movement of 4 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm. In this experiment, we did not include a water-filled glove and only ultrasound gel was added between the rabbit's legs. The purpose of not including a water-filled glove was to check the possibility of the ultrasound beam to reach the other inner side of the other leg (which it did not sonicated) and create lesion. For that reason, only the left thigh of the rabbit was sonicated.

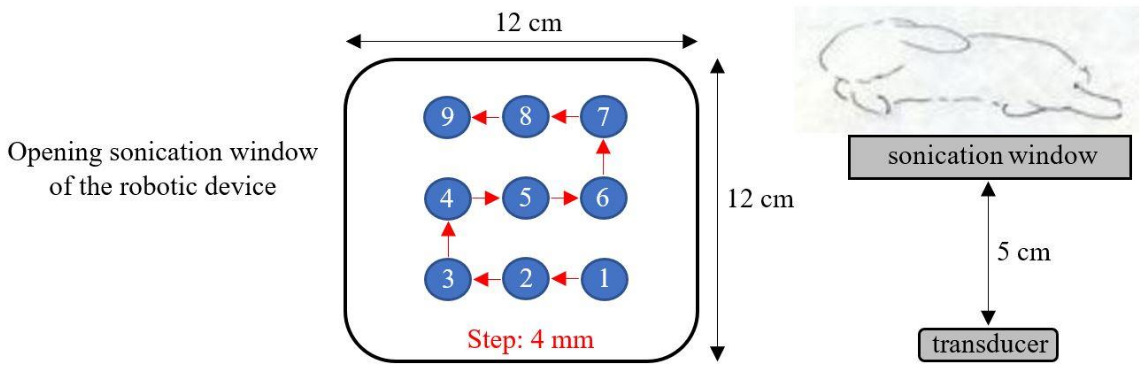

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 2 shows the experimental set-up in the laboratory, that was used for the rabbit thigh ablations.

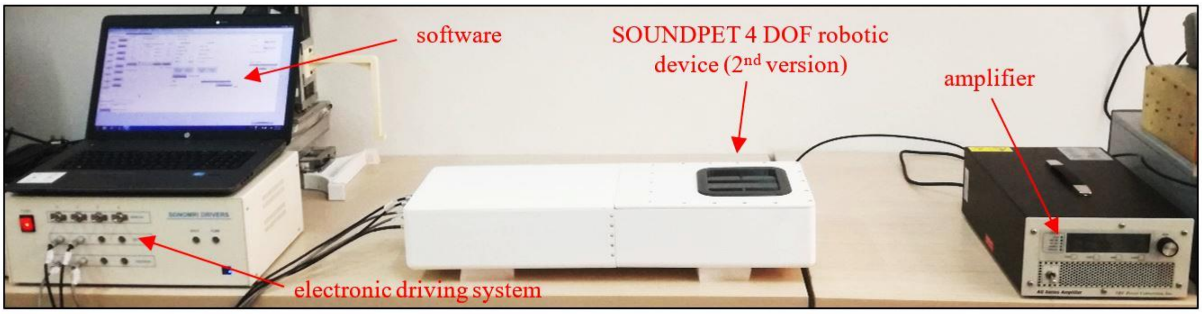

Figure 2: Experimental set-up for rabbit thigh ablation.

Figure 3 shows the anesthetized rabbit positioning for thigh ablation.

Figure 3: Animal positioning for the experiment.

Results

A necrotic area was appeared on the outer side (sonication side) of the left thigh above and below the skin as shown in Figure 4 and Figure 5, respectively.

Figure 4: The outer side of the left thigh above the skin after the treatment. The red arrow indicates the necrotic area on the skin (electric power 250 W for 5 s).

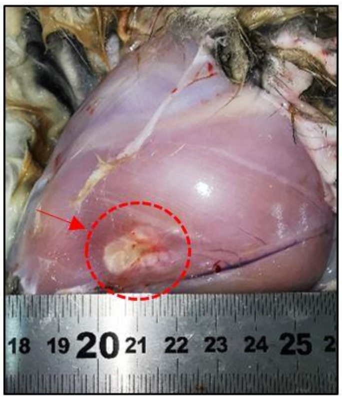

Figure 5: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side). The red arrow indicates the necrotic area (electric power 250 W for 5 s).

Necrotic area was observed on the inner side of the left thigh above the skin as shown in Figure 6 while two discrete lesions were observed below the skin as shown in Figure 7.

Figure 6: Macroscopic appearance of the inner side of the left thigh and above the skin. The red arrow indicates the necrotic area that was appeared on the skin (electric power 250 W for 5 s).

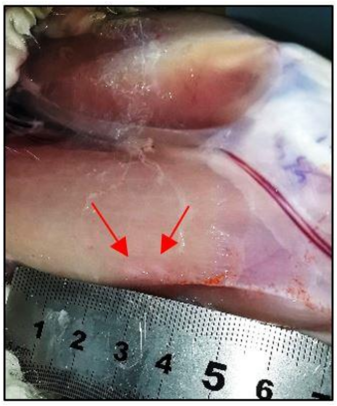

Figure 7: Macroscopic appearance of the exposed muscle from the inner side of the left thigh. The red arrows indicate the discrete lesions that were appeared on the exposed muscle (electric power 250 W for 5 s).

The muscle of the left thigh was dissected and a necrotic region was observed as shown in Figure 8.

Figure 8: Macroscopic appearance of the overlapping lesions (indicated with red arrow, electric power 250 W for 5 s) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 9 overlapping lesions on the surface of the muscle was calculated to be 7 mm x 11 mm while its depth was 16 mm. Figure 9 demonstrates the vertical cross middle dissection of the necrotic area (with its mirror view) that was created on the left thigh by applying a 3 x 3 grid pattern.

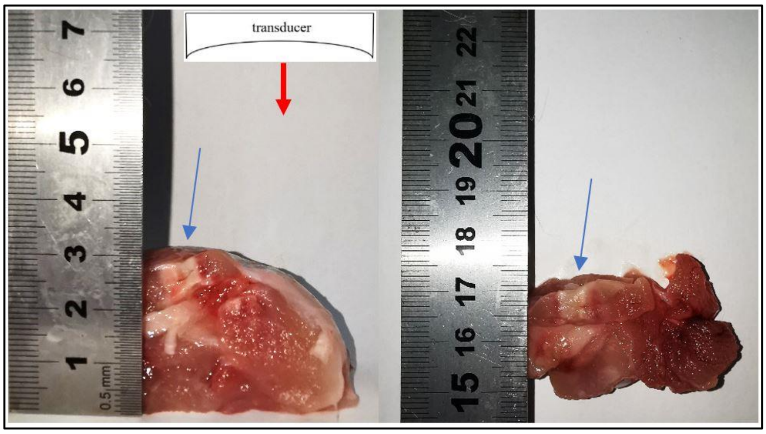

Figure 9: In situ dissection of the overlapping lesions that were created on the left thigh (electric power 250 W for 5 s). The blue arrows indicate the necrotic area and the red arrow indicates the beam direction.

Table 2 lists the anesthesia record for the rabbit experiment.

Table 2: Anesthesia record for rabbit 35.

Animal ID	SOUNDPET_2020_35
Species	Rabbit
Sex	M
Age	Adult
Weight (Kg)	3.00
Supplier	VET EX MACHINA
Grant	SOUNDPET (INTEGRATED/0918/0008)

Study	CY/EXP/PR.L01/2020
Participants	Kyriakos Spanoudes
	Theocharis Drakos
	Nikolas Evripidou
Principal Investigator	Christakis Damianou
Time	**Anesthesia**
11:25	Medetomidine 1.5 ml + Ketamine 0.5 ml
11:30	Midazolam 0.5 ml
Euthanasia	11:55 1 ml T-61

Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
11:30	100	62	0	Absent	n/a
11:40	96	66	0	Absent	n/a
11:50	102	64	0	Absent	n/a

Rabbit 36

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	250
Acoustic power (W)	57.5
Sonication time (s)	3
Energy (J)	172.5
Cooling time (s)	60
Number of sonications	9
Step between sonications (mm)	3
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 9 (direction from rabbit feet to tail) at step movement of 3 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm. In this experiment, we did not include a water-filled glove and only ultrasound gel was added between the rabbit's legs. The purpose of not including a water-filled glove was to check the possibility of the ultrasound beam to reach the other inner side of the other leg (which it did not sonicated) and create lesion. For that reason, only the left thigh of the rabbit was sonicated.

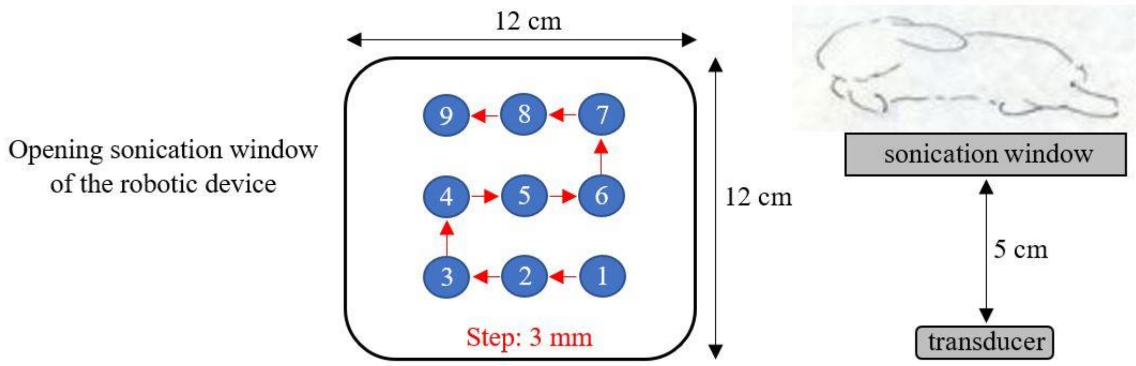

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 2 shows the experimental set-up in the laboratory, that was used for the rabbit thigh ablations.

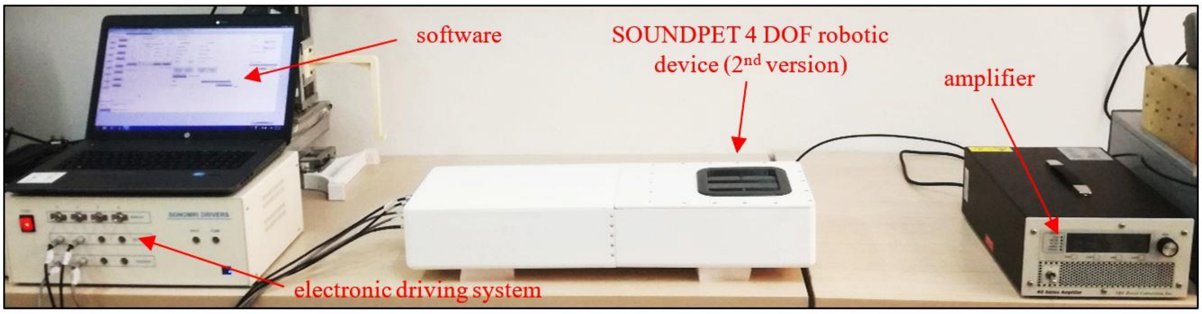

Figure 2: Experimental set-up for rabbit thigh ablation.

Figure 3 shows the anesthetized rabbit positioning for thigh ablation.

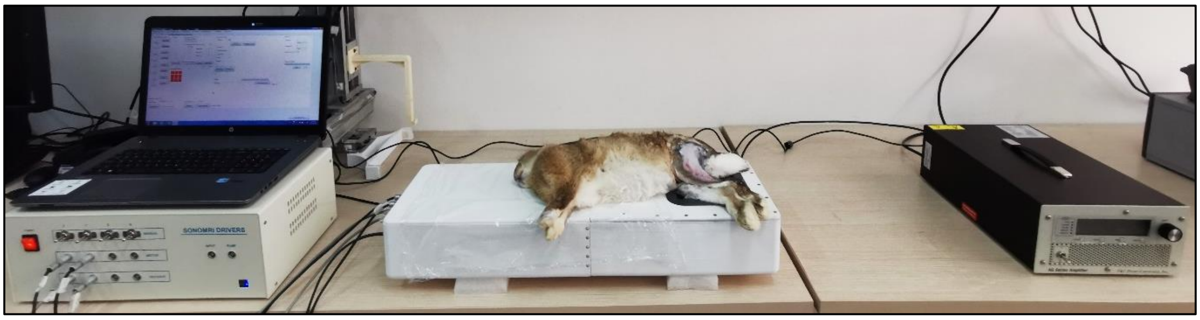

Figure 3: Animal positioning for the experiment.

Results

A necrotic area was appeared on the outer side (sonication side) of the left thigh above and below the skin as shown in Figure 4 and Figure 5, respectively.

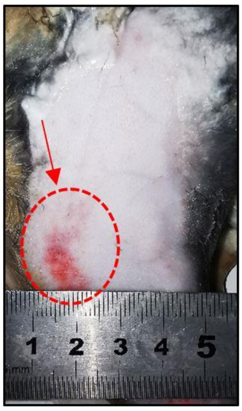

Figure 4: The outer side of the left thigh above the skin after the treatment. The red arrow indicates the necrotic area on the skin (electric power 250 W for 3 s).

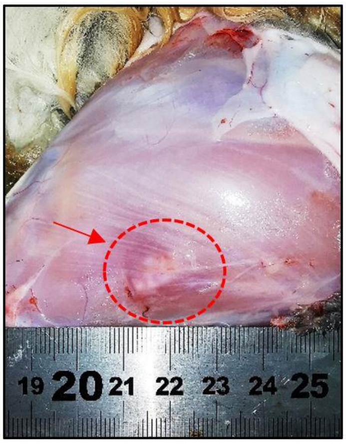

Figure 5: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side). The red arrow indicates the necrotic area (electric power 250 W for 3 s).

Necrotic area was observed on the inner side of the left thigh above and below the skin as shown in Figure 6 and Figure 7, respectively.

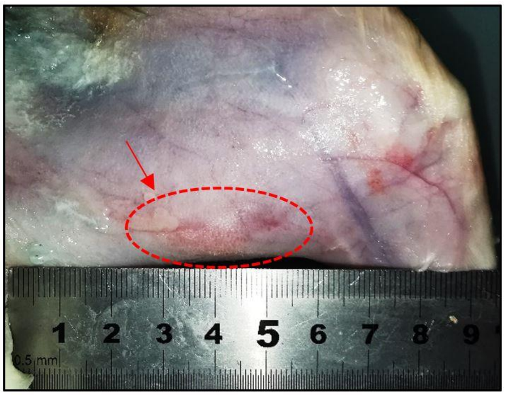

Figure 6: Macroscopic appearance of the inner side of the left thigh and above the skin. The red arrow indicates the necrotic area that was appeared on the skin (electric power 250 W for 3 s).

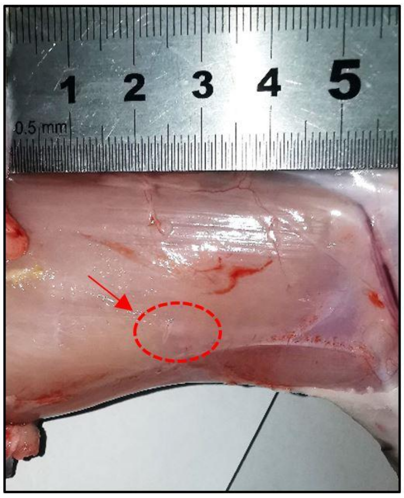

Figure 7: Macroscopic appearance of the exposed muscle from the inner side of the left thigh. The red arrows indicate the discrete lesions that were appeared on the exposed muscle (electric power 250 W for 3 s).

The muscle of the left thigh was dissected and a necrotic region was observed as shown in Figure 8.

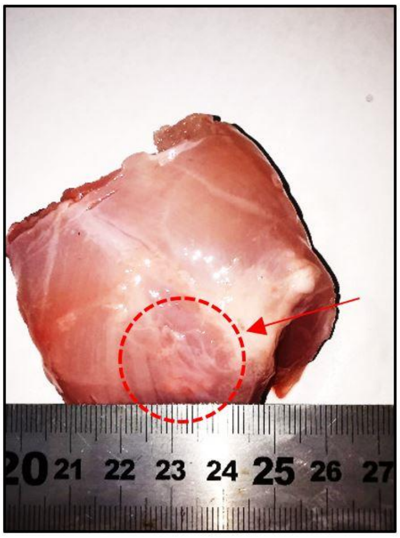

Figure 8: Macroscopic appearance of the overlapping lesions (indicated with red arrow, electric power 250 W for 3 s) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 9 overlapping lesions on the surface of the muscle was calculated to be 12 mm x 12 mm while its depth was 5 mm. Figure 9 demonstrates the vertical cross middle dissection of the necrotic area (with its mirror view) that was created on the left thigh by applying a 3 x 3 grid pattern.

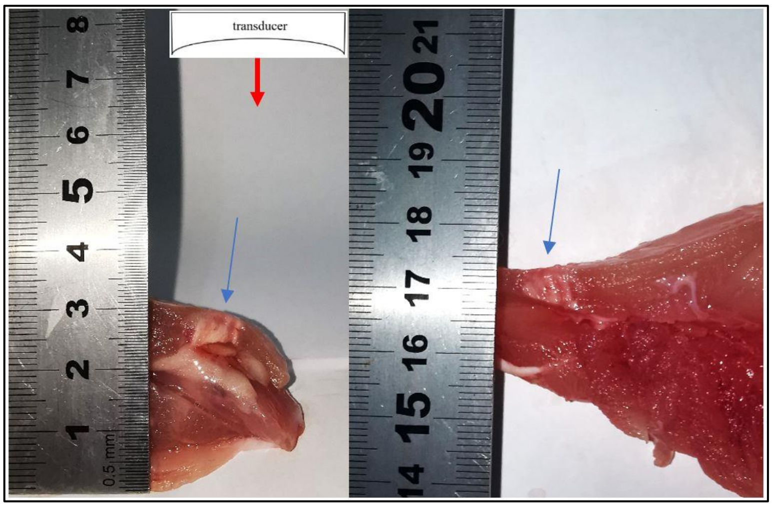

Figure 9: In situ dissection of the overlapping lesions that were created on the left thigh (electric power 250 W for 3 s). The blue arrows indicate the necrotic area and the red arrow indicates the beam direction.

Table 2 lists the anesthesia record for the rabbit experiment.

Table 2: Anesthesia record for rabbit 36.

Animal ID	SOUNDPET_2020_36
Species	Rabbit
Sex	F
Age	Adult
Weight (Kg)	2.85
Supplier	VET EX MACHINA
Grant	SOUNDPET (INTEGRATED/0918/0008)
Study	CY/EXP/PR.L01/2020
Participants	Kyriakos Spanoudes
	Theocharis Drakos
	Nikolas Evripidou
Principal Investigator	Christakis Damianou
Time	**Anesthesia**
11:40	Medetomidine 1.5 ml + Ketamine 0.5 ml
11:45	Midazolam 0.5 ml
Euthanasia	12:15 1 ml T-61

Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
11:50	110	68	0	Absent	n/a
12:00	100	68	0	Absent	n/a
12:10	100	62	1	Absent	n/a

Rabbit 37

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	300
Acoustic power (W)	69
Sonication time (s)	5
Energy (J)	345
Cooling time (s)	60
Number of sonications	9
Step between sonications (mm)	4
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 9 (direction from rabbit feet to tail) at step movement of 4 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm. In this experiment, we did not include a water-filled glove and only ultrasound gel was added between the rabbit's legs. The purpose of not including a water-filled glove was to check the possibility of the ultrasound beam to reach the other inner side of the other leg (which it did not sonicated) and create lesion. For that reason, only the left thigh of the rabbit was sonicated.

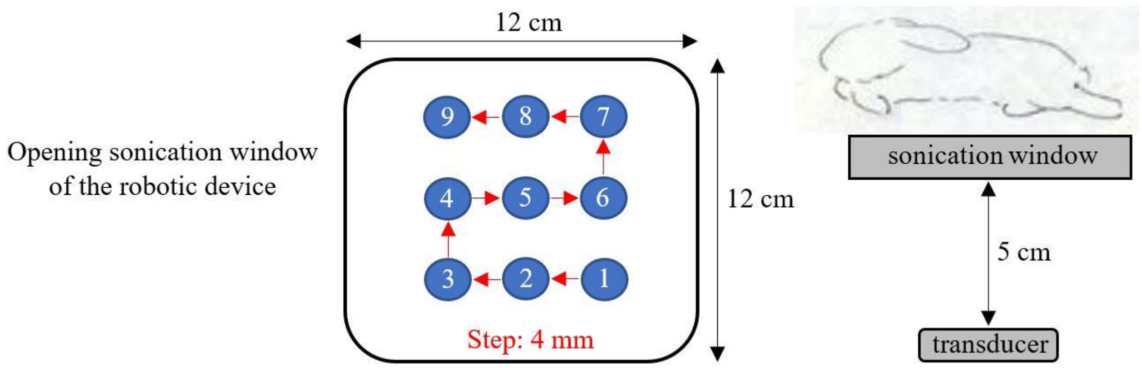

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 2 shows the experimental set-up in the laboratory, that was used for the rabbit thigh ablations.

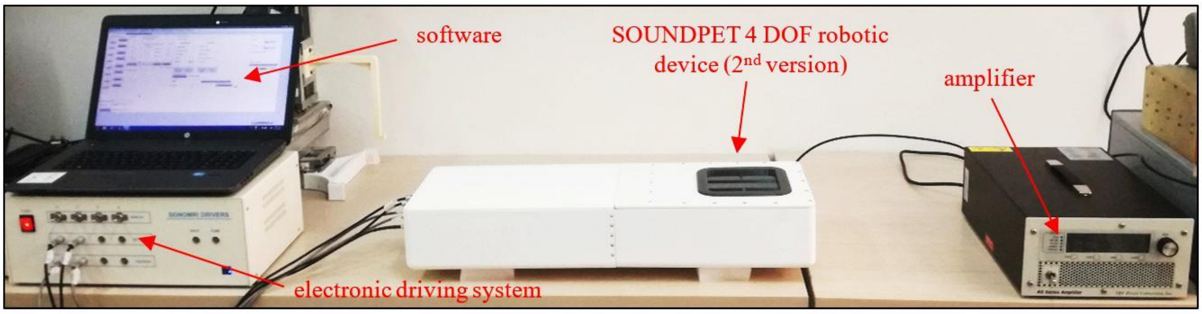

Figure 2: Experimental set-up for rabbit thigh ablation.

Figure 3 shows the anesthetized rabbit positioning for thigh ablation.

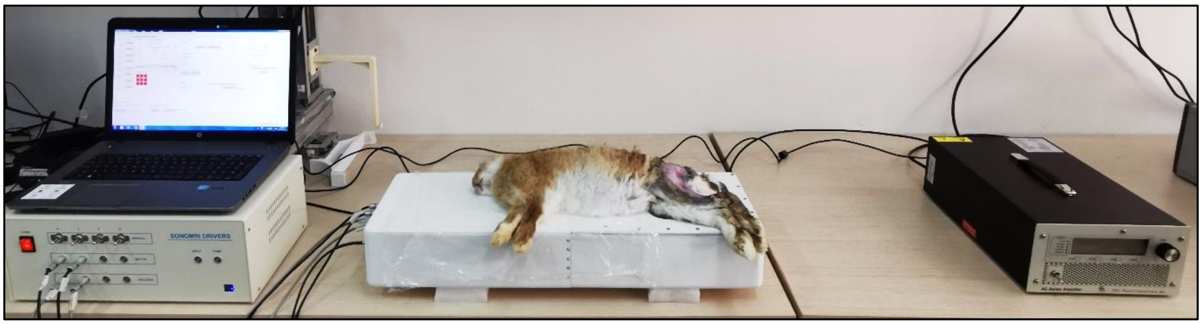

Figure 3: Animal positioning for the experiment.

Results

A necrotic area was appeared on the outer side (sonication side) of the left thigh above and below the skin as shown in Figure 4 and Figure 5, respectively.

Figure 4: The outer side of the left thigh above the skin after the treatment. The red arrow indicates the necrotic area on the skin (electric power 300 W for 5 s).

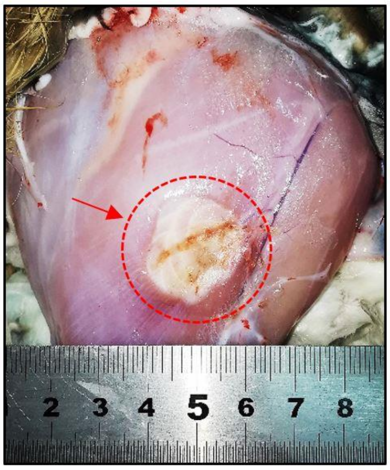

Figure 5: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side). The red arrow indicates the necrotic area (electric power 300 W for 5 s).

No lesions were observed on the inner side of the left thigh above and below the skin as shown in Figure 6 and Figure 7, respectively.

Figure 6: Macroscopic appearance of the inner side of the left thigh and above the skin. No lesions were observed on the skin (electric power 300 W for 5 s).

Figure 7: Macroscopic appearance of the exposed muscle from the inner side of the left thigh. No lesions were observed after the muscle exposure (electric power 300 W for 5 s).

The muscle of the left thigh was dissected and a necrotic region was observed as shown in Figure 8.

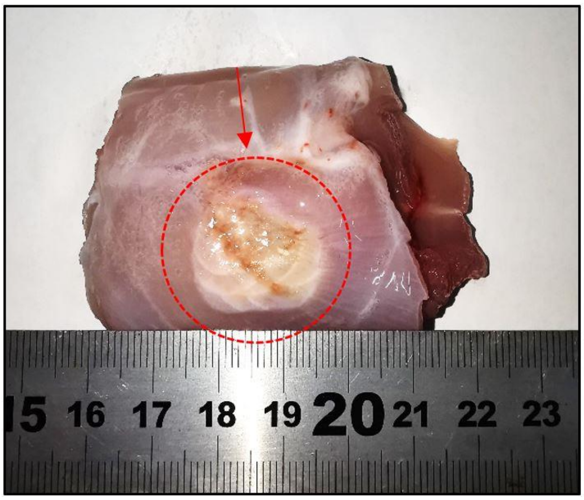

Figure 8: Macroscopic appearance of the overlapping lesions (indicated with red arrow, electric power 300 W for 5 s) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 9 overlapping lesions on the surface of the muscle was calculated to be 20 mm x 18 mm while its depth was 12 mm. Figure 9 demonstrates the vertical cross middle dissection of the necrotic area (with its mirror view) that was created on the left thigh by applying a 3 x 3 grid pattern.

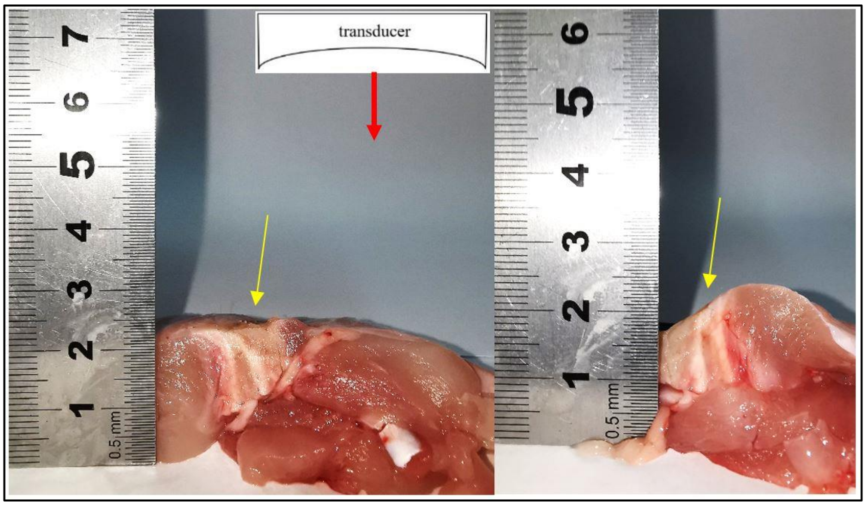

Figure 9: In situ dissection of the overlapping lesions that were created on the left thigh (electric power 300 W for 5 s). The yellow arrows indicate the necrotic area and the red arrow indicates the beam direction.

Table 2 lists the anesthesia record for the rabbit experiment.

Table 2: Anesthesia record for rabbit 37.

Animal ID	SOUNDPET_2020_37				
Species	Rabbit				
Sex	M				
Age	Adult				
Weight (Kg)	3.05				
Supplier	VET EX MACHINA				
Grant	SOUNDPET (INTEGRATED/0918/0008)				
Study	CY/EXP/PR.L01/2020				
Participants	Kyriakos Spanoudes				
	Theocharis Drakos				
	Nikolas Evripidou				
Principal Investigator	Christakis Damianou				
Time	**Anesthesia**				
12:35	Medetomidine 1.5 ml + Ketamine 0.5 ml				
12:40	Midazolam 0.5 ml				
Euthanasia	13:15 1 ml T-61				
Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
12:40	120	62	0	Absent	n/a
12:50	120	68	1	Absent	n/a
13:00	110	62	0	Absent	n/a
13:10	110	64	0	Absent	n/a

Rabbit 38

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	300
Acoustic power (W)	69
Sonication time (s)	3
Energy (J)	207
Cooling time (s)	60
Number of sonications	16
Step between sonications (mm)	3
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 16 (direction from rabbit feet to tail) at step movement of 3 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm. In this experiment, we did not include a water-filled glove and only ultrasound gel was added between the rabbit's legs. The purpose of not including a water-filled glove was to check the possibility of the ultrasound beam to reach the other inner side of the other leg (which it did not sonicated) and create lesion. For that reason, only the left thigh of the rabbit was sonicated.

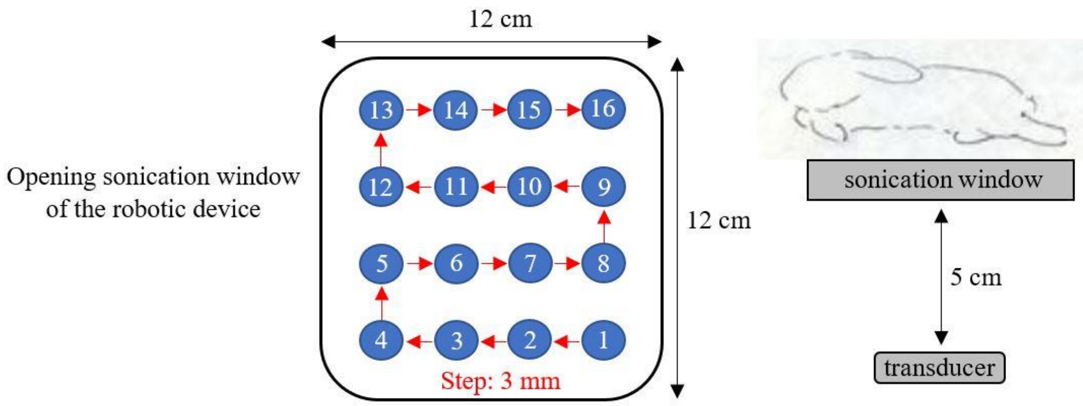

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 2 shows the experimental set-up in the laboratory, that was used for the rabbit thigh ablations.

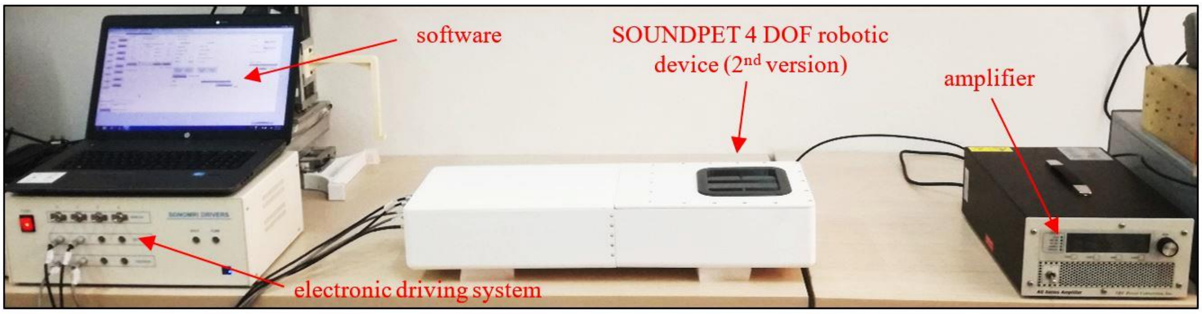

Figure 2: Experimental set-up for rabbit thigh ablation.

Figure 3 shows the anesthetized rabbit positioning for thigh ablation.

Figure 3: Animal positioning for the experiment.

Results

A necrotic area was appeared on the outer side (sonication side) of the left thigh above and below the skin as shown in Figure 4 and Figure 5, respectively.

Figure 4: The outer side of the left thigh above the skin after the treatment. The red arrow indicates the necrotic area on the skin (electric power 300 W for 3 s).

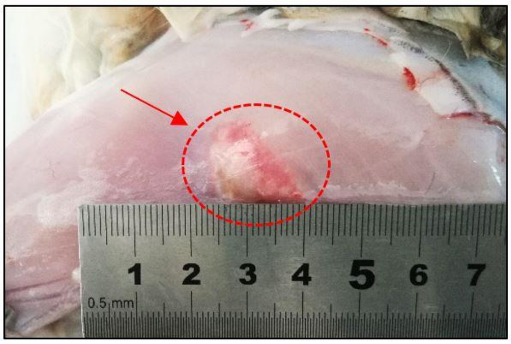

Figure 5: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side). The red arrow indicates the necrotic area (electric power 300 W for 3 s).

A faint necrotic area was observed on the inner side of the left thigh above the skin as shown in Figure 6 while no lesions were observed below the skin as shown in Figure 7.

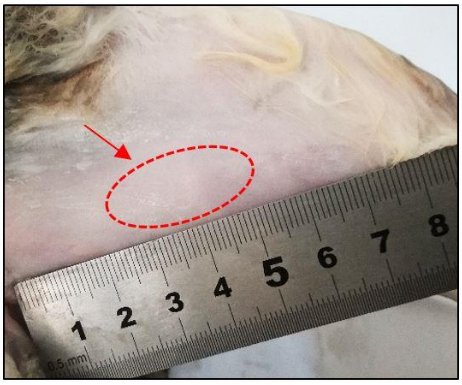

Figure 6: Macroscopic appearance of the inner side of the left thigh and above the skin. The red arrow indicates the faint necrotic area that was appeared on the skin (electric power 300 W for 3 s).

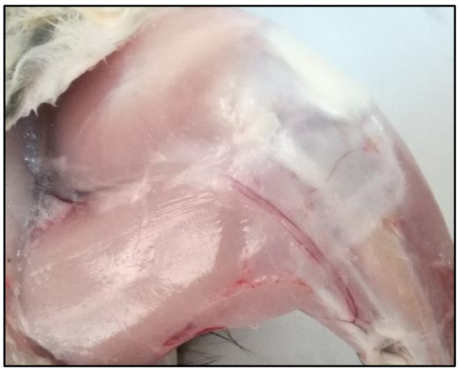

Figure 7: Macroscopic appearance of the exposed muscle from the inner side of the left thigh. No lesions were appeared on the exposed muscle (electric power 300 W for 3 s).

The muscle of the left thigh was dissected and a necrotic region was observed as shown in Figure 8.

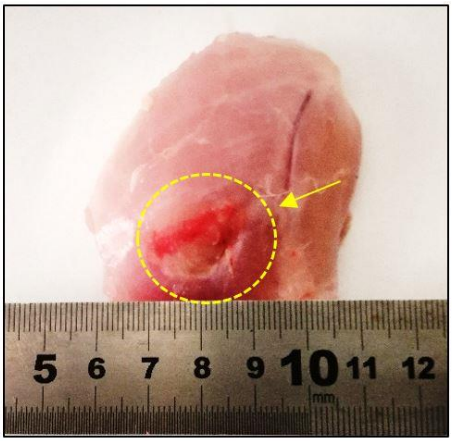

Figure 8: Macroscopic appearance of the overlapping lesions (indicated with yellow arrow, electric power 300 W for 3 s) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 16 overlapping lesions on the surface of the muscle was calculated to be 16 mm x 14 mm while its depth was 18 mm. Figure 9 demonstrates the vertical cross middle dissection of the necrotic area (with its mirror view) that was created on the left thigh by applying a 4 x 4 grid pattern.

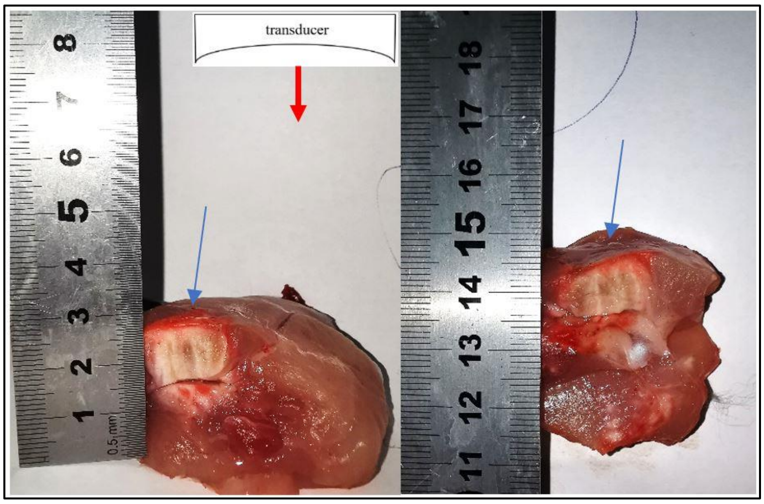

Figure 9: In situ dissection of the overlapping lesions that were created on the left thigh (electric power 300 W for 3 s). The blue arrows indicate the necrotic area and the red arrow indicates the beam direction.

Table 2 lists the anesthesia record for the rabbit experiment.

Table 2: Anesthesia record for rabbit 38.

Animal ID	SOUNDPET_2020_38
Species	Rabbit
Sex	M
Age	Adult

Weight (Kg)	3.00
Supplier	VET EX MACHINA
Grant	SOUNDPET (INTEGRATED/0918/0008)
Study	CY/EXP/PR.L01/2020
Participants	Kyriakos Spanoudes
	Theocharis Drakos
	Nikolas Evripidou
Principal Investigator	Christakis Damianou
Time	**Anesthesia**
13:15	Medetomidine 1.5 ml + Ketamine 0.5 ml
13:20	Midazolam 0.5 ml
Euthanasia	14:00 1 ml T-61

Time	**Heart Rate**	**Resp. Rate**	**Urination** (0 to 3)	**Absence of Movement /Pedal Reflex**	**Temp. (°C)**
13:20	110	68	1	Absent	n/a
13:30	100	68	0	Absent	n/a
13:40	110	62	0	Absent	n/a
13:50	110	64	1	Absent	n/a

Rabbit 39

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	300
Acoustic power (W)	69
Sonication time (s)	3
Energy (J)	207
Cooling time (s)	60
Number of sonications	25
Step between sonications (mm)	3
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 25 (direction from rabbit feet to tail) at step movement of 3 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm. In this experiment, we did not include a water-filled glove and only ultrasound gel was added between the rabbit's legs. The purpose of not including a water-filled glove was to check the possibility of the ultrasound beam to reach the other inner side of the other leg (which it did not sonicated) and create lesion. For that reason, only the left thigh of the rabbit was sonicated.

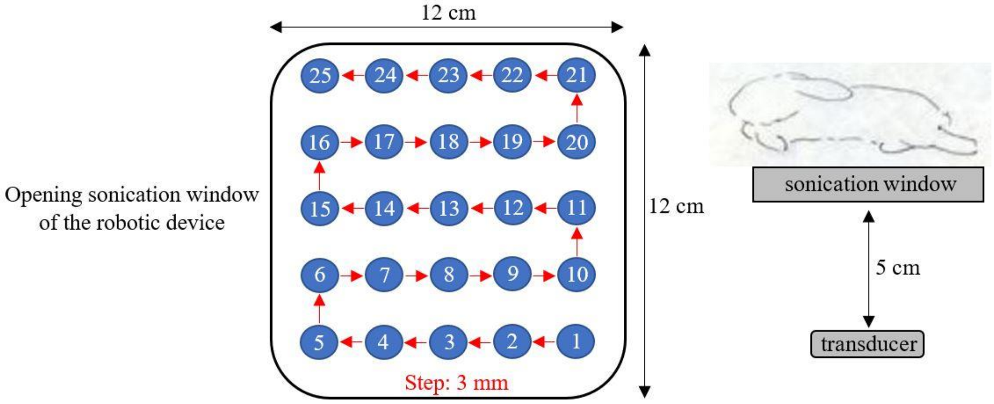

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 2 shows the experimental set-up in the laboratory, that was used for the rabbit thigh ablations.

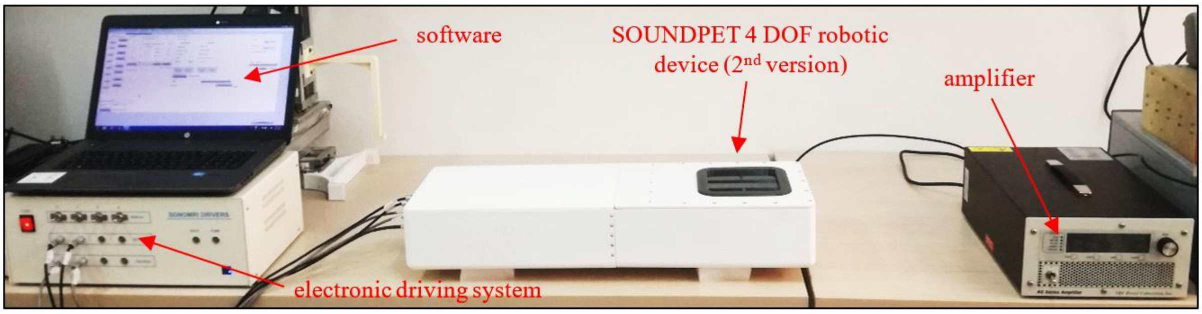

Figure 2: Experimental set-up for rabbit thigh ablation.

Figure 3 shows the anesthetized rabbit positioning for thigh ablation.

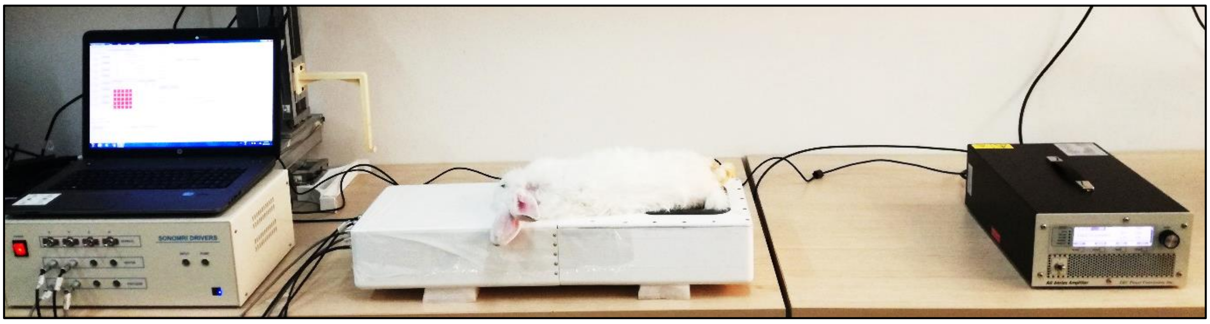

Figure 3: Animal positioning for the experiment.

Results

A necrotic area was appeared on the outer side (sonication side) of the left thigh above and below the skin as shown in Figure 4 and Figure 5, respectively.

Figure 4: The outer side of the left thigh above the skin after the treatment. The red arrow indicates the necrotic area on the skin (electric power 300 W for 3 s).

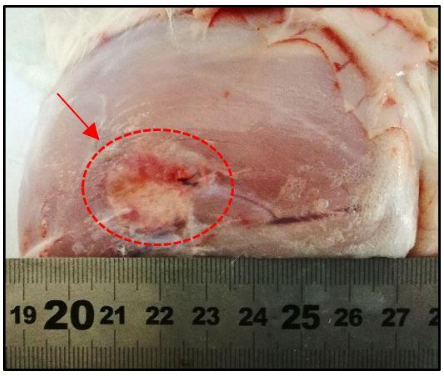

Figure 5: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side). The red arrow indicates the necrotic area (electric power 300 W for 3 s).

A faint necrotic area was observed on the inner side of the left thigh above the skin as shown in Figure 6 while no lesions were observed below the skin as shown in Figure 7.

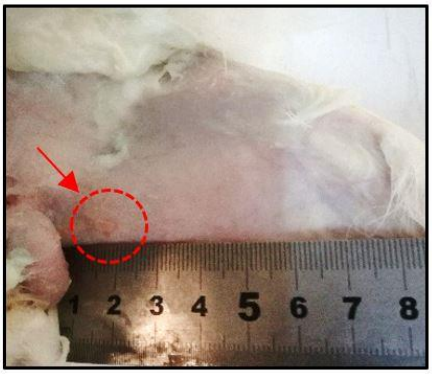

Figure 6: Macroscopic appearance of the inner side of the left thigh and above the skin. The red arrow indicates the faint necrotic area that was appeared on the skin (electric power 300 W for 3 s).

Figure 7: Macroscopic appearance of the exposed muscle from the inner side of the left thigh. The red arrows indicate the discrete lesions that were appeared on the exposed muscle (electric power 300 W for 3 s).

The muscle of the left thigh was dissected and a necrotic region was observed as shown in Figure 8.

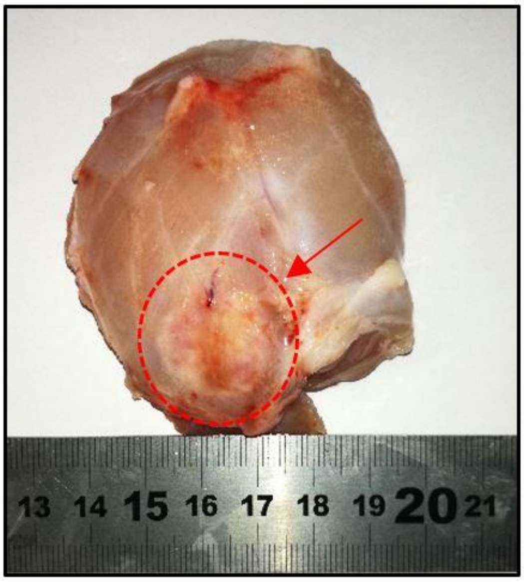

Figure 8: Macroscopic appearance of the overlapping lesions (indicated with red arrow, electric power 300 W for 3 s) on the dissected muscle of the inner side of the left thigh (plane perpendicular to the beam).

The area of the necrotic region that was created by 25 overlapping lesions on the surface of the muscle was calculated to be 20 mm x 20 mm while its depth was 8 mm. Figure 9 demonstrates the vertical cross middle dissection of the necrotic area (with its mirror view) that was created on the left thigh by applying a 5 x 5 grid pattern.

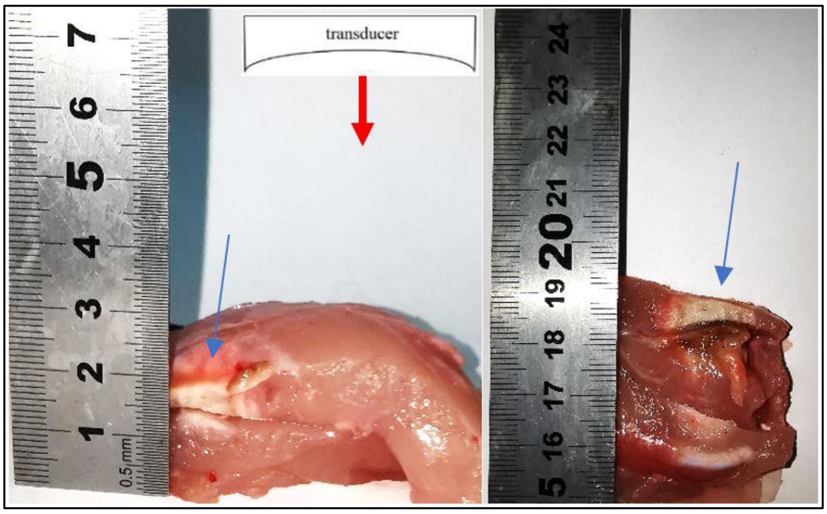

Figure 9: In situ dissection of the overlapping lesions that were created on the left thigh (electric power 300 W for 3 s). The blue arrows indicate the necrotic area and the red arrow indicates the beam direction.

Table 2 lists the anesthesia record for the rabbit experiment.

Table 2: Anesthesia record for rabbit 39.

Animal ID	SOUNDPET_2020_39
Species	Rabbit
Sex	M
Age	Adult
Weight (Kg)	2.87
Supplier	VET EX MACHINA
Grant	SOUNDPET (INTEGRATED/0918/0008)
Study	CY/EXP/PR.L01/2020
Participants	Kyriakos Spanoudes
	Theocharis Drakos
	Nikolas Evripidou
Principal Investigator	Christakis Damianou
Time	**Anesthesia**
14:40	Medetomidine 1.5 ml + Ketamine 0.5 ml
14:45	Midazolam 0.5 ml
Euthanasia	15:35 1 ml T-61

Time	Heart Rate	Resp. Rate	Urination (0 to 3)	Absence of Movement /Pedal Reflex	Temp. (°C)
15:00	110	68	0	Absent	n/a
15:10	110	68	0	Absent	n/a
15:20	100	72	0	Absent	n/a
15:30	110	68	2	Absent	n/a

Rabbit 40

Place: Therapeutic Ultrasound Laboratory (CUT)

Participants: K. Spanoudes, T. Drakos, N. Evripidou

Settings for thigh ablations

Table 1 lists the settings that were used for the sonications on the left thigh.

Table 1: Settings for left thigh.

Parameter	Value
Electric power (W)	300
Acoustic power (W)	69
Sonication time (s)	5
Energy (J)	345
Cooling time (s)	60
Number of sonications	25
Step between sonications (mm)	3
Distance transducer/rabbit skin (cm)	5

A linear grid was chosen for the left thigh ablations as shown in Figure 1. The robotic device was moved from point 1 to 25 (direction from rabbit feet to tail) at step movement of 3 mm and the distance between the transducer and the opening sonicated window of the robotic device was 5 cm. In this experiment, we did not include a water-filled glove and only ultrasound gel was added between the rabbit's legs. The purpose of not including a water-filled glove was to check the possibility of the ultrasound beam to reach the other inner side of the other leg (which it did not sonicated) and create lesion. For that reason, only the left thigh of the rabbit was sonicated.

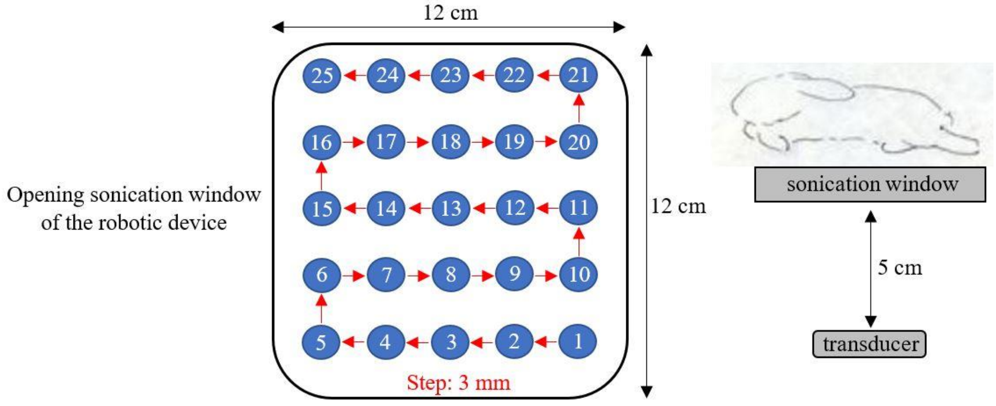

Figure 1: Grid movement for ablations on the left thigh and the distance transducer/rabbit thigh.

Experimental set-up

Figure 2 shows the experimental set-up in the laboratory, that was used for the rabbit thigh ablations.

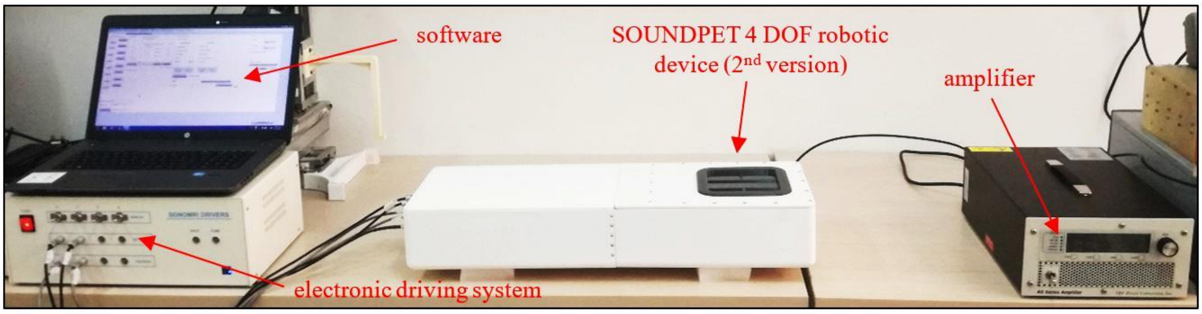

Figure 2: Experimental set-up for rabbit thigh ablation.

Figure 3 shows the anesthetized rabbit positioning for thigh ablation.

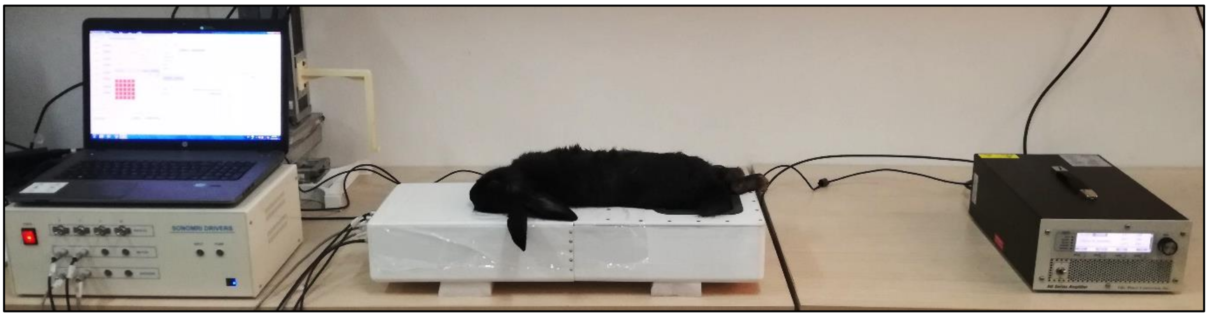

Figure 3: Animal positioning for the experiment.

Results

A necrotic area was appeared on the outer side (sonication side) of the left thigh above and below the skin as shown in Figure 4 and Figure 5, respectively.

Figure 4: The outer side of the left thigh above the skin after the treatment. The red arrow indicates the necrotic area on the skin (electric power 300 W for 5 s).

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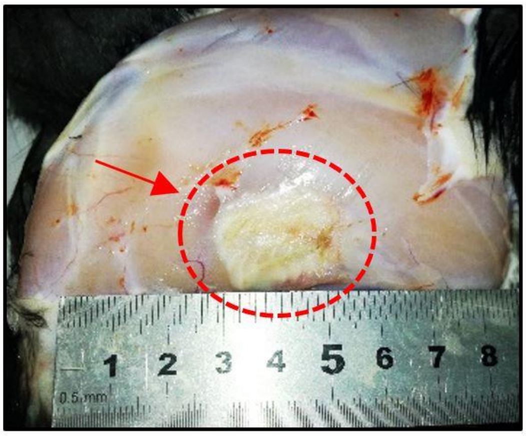

Figure 5: The outer side of the left thigh when the skin was removed and the muscle was exposed (sonication side). The red arrow indicates the necrotic area (electric power 300 W for 5 s).

No lesions were observed on the inner side of the left thigh above the skin as shown in Figure 6. However, a necrotic area was observed below the skin as shown in Figure 7.

Figure 6: Macroscopic appearance of the inner side of the left thigh and above the skin. No lesions were appeared on the skin (electric power 300 W for 5 s).

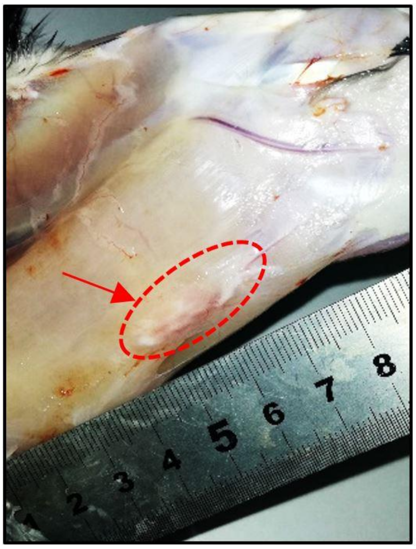

Figure 7: Macroscopic appearance of the exposed muscle from the inner side of the left thigh. The red arrow indicates the necrotic area that was appeared on the exposed muscle (electric power 300 W for 5 s).

The area of the necrotic region that was created by 25 overlapping lesions on the surface of the muscle was calculated to be 24 mm x 20 mm while its depth was 7 mm. Figure 8 demonstrates the vertical cross middle dissection of the necrotic area that was created on the left thigh by applying a 5 x 5 grid pattern.

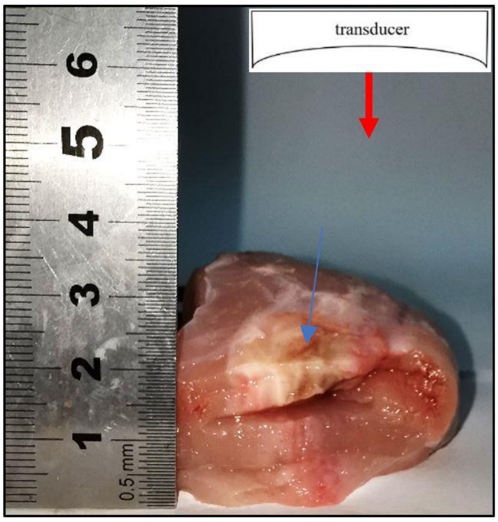

Figure 8: In situ dissection of the overlapping lesions that were created on the left thigh (electric power 300 W for 5 s). The blue arrows indicate the necrotic area and the red arrow indicates the beam direction.

Table 2 lists the anesthesia record for the rabbit experiment.

Table 2: Anesthesia record for rabbit 40.

<table>
<tr><td colspan="3">Animal ID</td><td colspan="3">SOUNDPET_2020_40</td></tr>
<tr><td colspan="3">Species</td><td colspan="3">Rabbit</td></tr>
<tr><td colspan="3">Sex</td><td colspan="3">M</td></tr>
<tr><td colspan="3">Age</td><td colspan="3">Adult</td></tr>
<tr><td colspan="3">Weight (Kg)</td><td colspan="3">3.15</td></tr>
<tr><td colspan="3">Supplier</td><td colspan="3">VET EX MACHINA</td></tr>
<tr><td colspan="3">Grant</td><td colspan="3">SOUNDPET (INTEGRATED/0918/0008)</td></tr>
<tr><td colspan="3">Study</td><td colspan="3">CY/EXP/PR.L01/2020</td></tr>
<tr><td colspan="3" rowspan="3">Participants</td><td colspan="3">Kyriakos Spanoudes</td></tr>
<tr><td colspan="3">Theocharis Drakos</td></tr>
<tr><td colspan="3">Nikolas Evripidou</td></tr>
<tr><td colspan="3">Principal Investigator</td><td colspan="3">Christakis Damianou</td></tr>
<tr><td colspan="6"></td></tr>
<tr><td colspan="3">Time</td><td colspan="3">Anesthesia</td></tr>
<tr><td colspan="3">15:30</td><td colspan="3">Medetomidine 1.5 ml + Ketamine 0.5 ml</td></tr>
<tr><td colspan="3">15:35</td><td colspan="3">Midazolam 0.5 ml</td></tr>
<tr><td colspan="3">Euthanasia</td><td colspan="3">16:15 1 ml T-61</td></tr>
<tr><td colspan="6"></td></tr>
<tr><td>Time</td><td>Heart Rate</td><td>Resp. Rate</td><td>Urination
(0 to 3)</td><td>Absence of Movement /Pedal Reflex</td><td>Temp. (°C)</td></tr>
</table>

15:40	100	72	1	Absent	n/a
15:50	98	64	0	Absent	n/a
16:00	110	68	0	Absent	n/a
16:10	100	68	0	Absent	n/a