Application of ultrasound to remove thrombi from the Left Ventricular Assist Device (LVAD)

Arundhati Radhakrishnan

Master of Science Thesis

TU Delft Delft University of Technology

Biomedical Electronics

Application of ultrasound to remove thrombi from the Left Ventricular Assist Device (LVAD)

Master of Science Thesis

For the degree of Master of Science in Biomedical Engineering at Delft University of Technology

Arundhati Radhakrishnan

September 7, 2015

Faculty of Mechanical, Maritime and Materials Engineering (3mE) · Delft University of Technology

The work in this thesis was supported by Erasmus Medical Centre. Their cooperation is hereby gratefully acknowledged.

Delft University of Technology
Department of
Biomedical Electronics

The undersigned hereby certify that they have read and recommend to the Faculty of Mechanical, Maritime and Materials Engineering (3mE) for acceptance a thesis entitled

Application of ultrasound to remove thrombi from the Left Ventricular Assist Device (LVAD)

by

Arundhati Radhakrishnan

in partial fulfillment of the requirements for the degree of

Master of Science Biomedical Engineering

Dated: September 7, 2015

Supervisor(s):

Prof.dr.ir. W.A. Serdijn

Prof.dr.ir. N. de Jong

Dr.ir. J.G. Bosch

Reader(s):

Dr.ir. C. Poelma

Abstract

Due to limited number of donor hearts and stringent eligibility criteria for heart transplant the Left ventricular assist device (LVAD) has emerged as a relevant treatment option for heart failure. Occlusion in the form of a thrombus (blood clot) is a feared complication associated with the LVAD. The ability of ultrasound to result in effects like cavitation, which is hypothesized to be one of the mechanisms contributing to sonothrombolysis(ultrasound mediated thrombolysis) forms the basis of proposing a solution wherein ultrasound is used to remove thrombi from the LVAD. The proposed solution entails catheter delivery of ultrasound into the LVAD to break down the thrombus.

In this master thesis an experimental setup to conduct sonothrombolysis tests on invitro clots has been realized. In order to understand the mechanism contributing to a high degree of sonothrombolysis a commonly used method - passive cavitation detection is also employed. In the final experiments sonothrombolysis and passive cavitation detection tests are conducted on two sets of 6 clots each. For majority of the clots, sonothrombolysis occurs at a peak negative pressure of 2.71MPa - 3.18 MPa. Clots which underwent a high degree of sonothrombolysis were always accompanied by high counts and violent movement. We assume the intermittent spikes termed as *cavitation events* being counted are due to physical effects like inertial bubble collapse, shockwaves and microjets, which are characteristic of inertial cavitation. Hence we can conclude that the high counts are indicative of inertial cavitation and play a dominant role in achieving a high degree of sonothrombolysis. The results of this master thesis provide experimental evidence as to why a certain threhold of peak negative pressure must be attained in order to achieve a high degree of sonothrombolysis. This evidence can be utilized in the next step of catheter design. At this stage it can be said that the application of ultrasound to remove thrombi from the LVAD will prove to be successful if high intensity ultrasound resulting in inertial cavitation can be delivered to the site of the thrombi formation in the LVAD.

Contents

List of Figures

List of Tables

Preface

This thesis concludes the Master of Science degree in Biomedical Engineering with a specialization in Biomedical Electronics.

The inception of this project is due to the collaboration between cardiologists Dr. J. Schaar, Dr. K. Caliskan and the Biomedical engineering department at the Erasmus Medical Centre. Together they wished to investigate whether it would be possible to remove thrombus (blood clots) from a Left ventricular assist device (LVAD) using ultrasound.

The Thoratech Heartmate II LVAD has been used during experiments conducted towards this thesis. Kees Van der Heiden provided the HeartMate II rotor and crucial information pertaining to thrombi formation in the LVAD.

The supervisors of this thesis have been Wouter Serdijn, Hans Bosch and Nico de Jong.

The initial phase of this project involved a literature review and a set of preliminary experiments. These were done in collaboration with colleague Maral Batgeral.

The experimental work conducted was done with technical assistance from Robert Beurskens.

The meetings with Heleen van Beusekom and Anoushka Atar from Experimental Cardiology were immensely useful in making the clots that have been used in the experiments conducted in this thesis. The clots were made from blood obtained from Experimental Cardiology. Andre Uitterdijk assisted while preparing the first batch of blood clots.

Acknowledgements

There are several key people I would like to thank for making this thesis a successful one. It would not have been possible without their support and I owe them my most sincere appreciation.

To begin with I would like to thank Wouter Serdijn for being my supervisor for this master thesis. There have been several occasions when I have been overwhelmed during this Masters programme, his friendly nature and objective criticism have helped me improve and move forward. Needless to say, I would not be here at the cusp of graduation had it not been for his support and guidance.

This thesis has been a steep learning curve for me and I was extremely fortunate to have Hans Bosch as my daily supervisor. In many ways he has been the ideal supervisor, always there to discuss and teach. I cannot thank him enough for the many hours he has spent guiding me throughout the various stages of this masters thesis.

I would also like to thank Nico de Jong for providing valuable feedback during the monthly meetings and for teaching me by asking me questions which forced me to think.

Thanks to Roberts Beurskens for always being friendly and helping me with the experimental setup (and introducing me to dutch liquorice).

Thanks to my colleagues Andre Uitterdijk, Sophinese Iskander-Rizk and Jovana Janjic for the discussions about my experiments.

I am grateful for all the friends I have made during my stay here at Delft. They have made my experience here one I will always cherish.

Last but certainly not the least, I am blessed to have a very loving and supportive family. My mother and husband have been my two pillars of strength encouraging me every single step of the way.

Delft, University of Technology — Arundhati Radhakrishnan
September 7, 2015

Glossary

List of Acronyms

STBL	Sonothrombolysis
PCD	Passive cavitation detection
HF	Heart failure
LVAD	Left ventricular assist device
DOT	Degree of thrombolysis
TIMI	Thrombolysis in Myocardial Infarction
CWL	Clot weight loss
RC	Recanalization
FFT	Fast fourier transform
HIFU	High intensity focussed ultrasound

Chapter 1

Introduction

1-1 Motivation

Heart failure (HF) is an end-stage heart condition in which the heart is too weak to pump blood to the entire body. It represents the end-stage of a number of cardiac diseases including ischemic heart disease, dilated cardiomyopathy and hypertensive heart disease [7]. HF is a wide spread problem affecting over 23 million people worldwide and over 10 million people in Europe alone [8].

Most patients with HF are treated pharmacologically. However, there are a set of patients where the HF has progressed to a state that is no longer manageable with medication, and these patients would ideally benefit from heart transplant. Due to limited number of donor hearts and stringent eligibility criteria for heart transplant the Left Ventricular Assist Device (LVAD) has emerged as a relevant treatment option [9].

The LVAD works by taking over the function of the damaged left ventricle and restores normal haemodynamics and end-organ blood flow [10]. It is composed of an inflow conduit, a pump, and an outflow graft. The inflow conduit of the LVAD is attached to the apex of the left ventricle and the outflow graft of the LVAD is attached to the ascending aorta creating an alternate route for blood flow as shown in Figure 1-1. The pump powers the flow of blood to the rest of the body thus relieving the strain on the heart to pump by itself.

However, it is not free of undesirable consequences. An infrequent but serious complication is the formation of a thrombus (blood clot) within the LVAD. The obstruction caused by a thrombus can severely occlude blood flow in a continuous flow device like the LVAD. This can lead to catastrophic events such as stroke, hemodynamic collapse and is associated with increased morbidity and mortality [4].

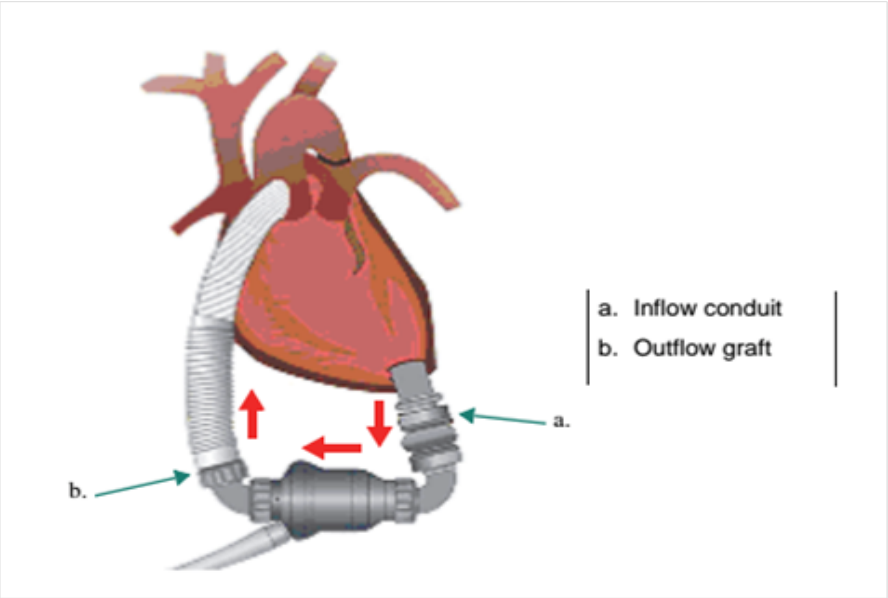

Figure 1-1: LVAD pump. Picture courtesy [1]

This master thesis lies in the domain of exploratory research wherein the emergence of the LVAD as the more accessible treatment option is recognized, and a solution to the complication of thrombus formation in the LVAD is investigated.

1-2 Pump thrombus - A feared complication

Haemostasis and thrombus formation

The human cardiovascular system is a closed, high pressure ciculatory system that maintains a state of equilibrium between an anticoagulant and procoagulant state. Haemostasis is a process by which damage to the blood wall is repaired via a series of reactions that results in the formation of a thrombus.

Thrombosis is generally classified into venous thrombosis and arterial thrombosis, both of which can have consequences that can be potentially fatal.

Virchows triad broadly describes the three factors that are thought to contribute to thrombosis: (i) Hypercoaguable state, (ii) Disruption in blood flow, (iii) Vessel wall injury/dysfunction [11].

This being said the cause for thrombus formation in the LVAD has not been investigated but have been hypothesized to be due to:

- Ingestion of material external to the pump: Post surgical ventricular debris, emboli

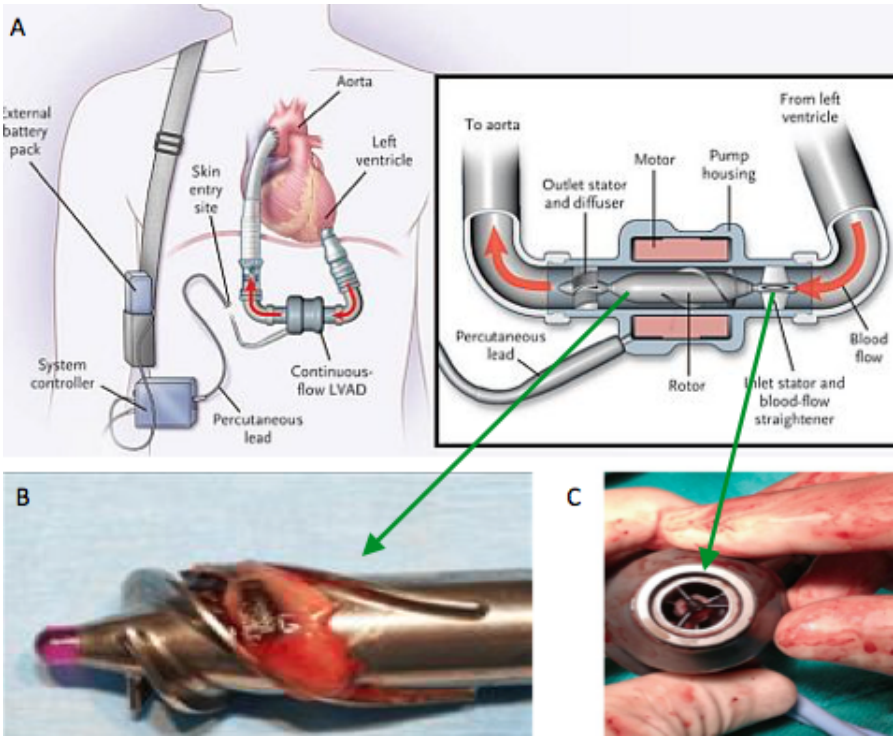

Figure 1-2: A: LVAD occlusion. Picture courtesy of [2], B: Clot on rotor on explanted rotor.Picture courtesy of [3], C: clot on inflow bearing of explanted LVAD. Picture courtesy of [3].

(piece of thrombus that has broken free) arising from left appendage, or from the endocardial surface of the left ventricle.

- Malposition of the inflow cannula resulting in obstruction of blood flow [4].

Location of pump thrombus

On explantation, the pump thrombus was most often found in the following locations within the LVAD.

- rotor, this can be seen in Figure 1-2 B
- inflow bearing, this can be seen in Figure 1-2 C

Clinical indicators of pump thrombus

For the pump thrombus to be treated, the physician must have an indicator that there is an occlusion due to which the LVAD is not performing its intended function. In the

study done by [4] the pump thrombus was defined as an event occurring > 72 hours after LVAD implantation and manifested as:

- an unexpected increase or sudden drop in pump parameters such as power and flow,
- increase in biochemical markers (LDH and plasma free haemoglobin) of hemolysis,
- visual observation of thrombi after explantation of the LVAD
- ausculatation of abnormal pump sounds

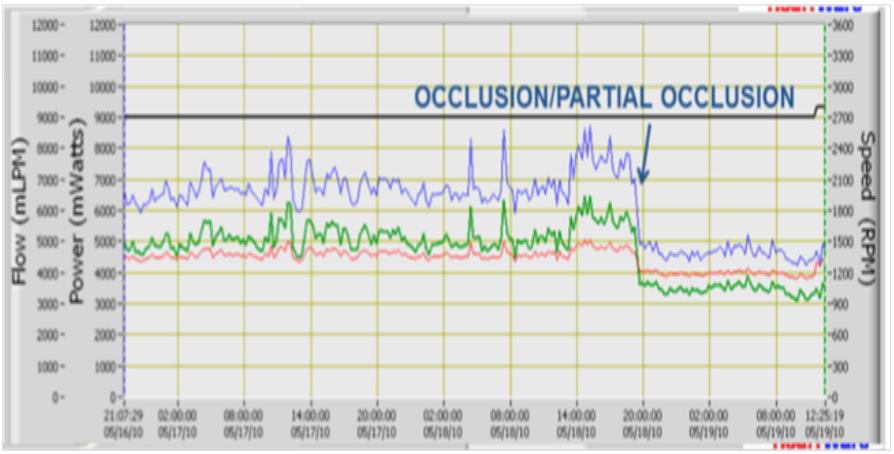

Figure 1-3: Log file tracings of power (red line), average controller flow (green line), and estimated flow (blue line) in total or subtotal occlusion.Picture courtesy of [4]

Furthermore the analysis of 34 pump thrombus events in 382 patients with the HeartWare LVAD [4] showed that a thrombus event exhibited different patterns of presentation:

- acute total or subtotal occlusion due to pump thrombus: precipitous drop in power and flow, this can be seen in Figure 1-3
- gradual thrombus formation (over 3 days): gradual rise in flow and power over days leading up to the thrombus event can be seen in Figure 1-4.

Treatment options for pump thrombus

Once these manifestations were observed, the treatment options for a suspected or confirmed pump thrombus was via pharmacological and/or surgical interventions.

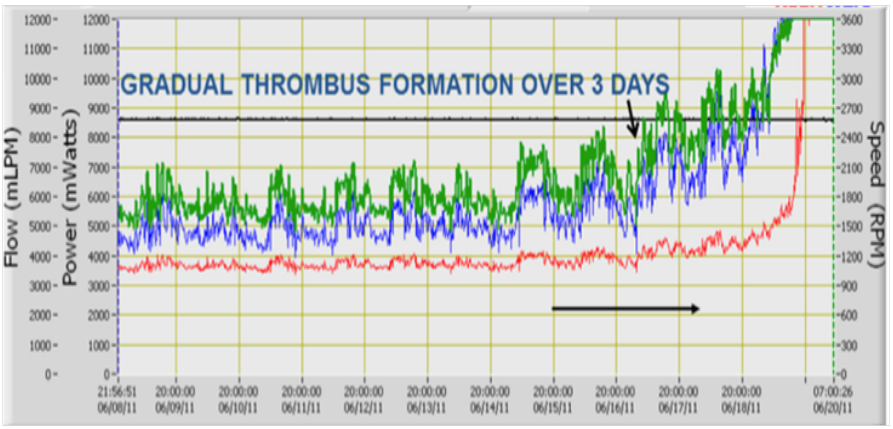

Figure 1-4: Log file tracings of power (red line), average controller flow (green line), and estimated flow (blue line) in a thrombus formed over several days.Picture courtesy of [4]

Pharmacological treatment

The pharmacological treatment consisted of unfractionated heparin, glycoprotein IIb/IIIa inhibitors and rtPA alone, or in combination. Analysis of the results of the treatment via pharmacological intervention do not look promising. There is still the risk recurrent thrombosis, and need for pump exchange [12]. In the HeartWare ADVANCE bridge to transplant and continued access protol trial, only 15 out of 30 (50%) patients were successfully treated with medical therapy alone. Out of the 30 patients who were treated medically, 5 experienced bleeding events, including 2 who suffered hemorrhagic cerebrovascular accidents and 3 patients experienced recurrent pump thrombosis [4]. In another study [13], that looked at the pump thrombus events in patients with the HeartMate II reported a mortality rate of 50% in patients who were treated only with pharmacological intervention.

Surgical treatment

Surgical intervention involves exchanging the thrombosed pump with a new one. In the study done by [13] pump exchange seems to have a better success rate as compared to pharmacological treatment with a mortality rate of 5% (1 out of 19) in patients who when compared to 15 out of 30 (50%).

However, device exchange for a suspected thrombosis is not inconsequential. The INTERMACS database reported a significant decrease in 2-year survival in patients who required a device exchange compared with survival seen with the initial implantation. The incidence of infection and neurological events were also significantly higher in patients who required a pump exchange [12].

1-3 Aim - Restoration of unoccluded blood flow in LVAD by removal of thrombi

The aim of this master thesis is to investigate the possibility of using ultrasound to remove thrombi from the LVAD. As can be seen in Figure 1-2 removal of the thrombi will restore unoccluded blood flow, ensuring proper functioning of the device such that it can perform its function of taking over the pumping action of the heart. The features of ultrasound that make it a viable means to achieve this aim are elaborated upon in the following section.

1-4 Ultrasound - Means to achieve the aim

Ultrasound physics

Ultrasound waves are mechanical pressure waves with frequencies greater than 20 kHz. These waves require a vibrating source and a medium to travel in. The mechanical movement of the vibrating source in the forward direction compresses the medium particles in front of it, increasing their concentration per unit volume, hence increasing pressure; this phase is known as compression. In the reverse direction the medium particles are decompressed giving rise to low pressure, this phase is known as rarefaction [5].

Ultrasound transducers - Two way transducer

As mentioned above ultrasound waves require a vibrating source. In ultrasound transducers this source is the piezoelectric element. This piezoelectric element can convert electrical signals into mechanical vibrations and mechanical vibrations into electrical signals. Due to this, these transducers have the interesting feature of being suitable as both a sensor and an actuator.

This feature of ultrasound transducers is being explained because in the experimental setup of this master thesis two ultrasound transducers are employed - one generating mechanical pressure waves (transmit mode) and the other detecting mechanical pressure waves (receive mode).

Ultrasound mediated thrombolysis - Sonothrombolysis (STBL)

The basic theory behind the generation of an ultrasound pressure wave has been explained. This basic theory of ultrasound was necessary in order to understand how the propagation of this ultrasound wave in liquid media results in certain effects which can lead to sonothrombolysis.

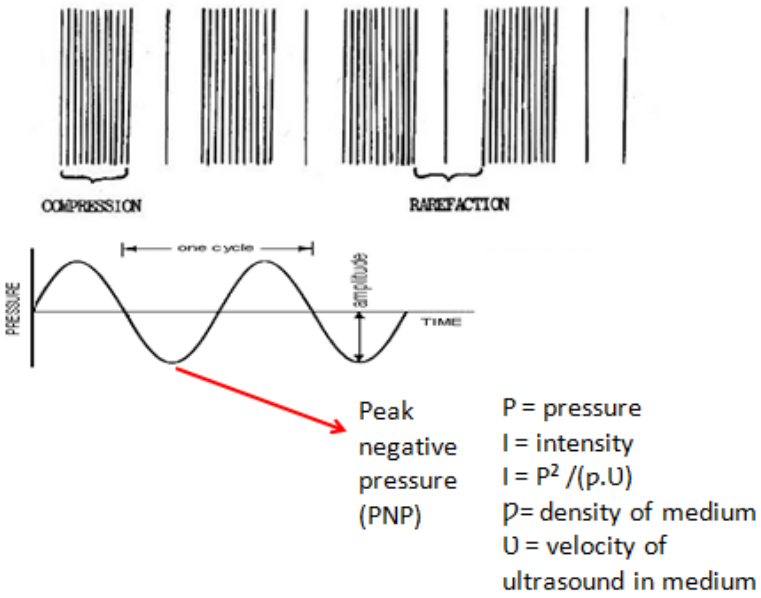

Figure 1-5: US wave exhibiting high pressure amplitude during compression and low pressure during rarefaction.Picture courtesy of [5]

Sonothrombolysis studies have been done since the 1970's, some of these studies employed thrombolytic drugs (rtPA) and/or microbubbles to enhance thrombolysis. Microbubbles are micron sized bubbles with a polymer or lipid coating filled with an easily compressible gas.

The most commonly hypothesized mechanisms via which ultrasound propagation in liquid media results in sonothrombolysis are briefly explained below:

Acoustic streaming

Ultrasound induces axial fluid acceleration that results in the phenomena of acoustic streaming, the high velocity gradients along and around a thrombus results in a mechanical shearing effect, which results in sonothrombolysis [14].

Cavitation

When ultrasound propagates through liquid media, the cyclic succession of compression and rarefaction pushes and pulls the liquid molecules together and apart. When the pressure amplitude exceeds the tensile strength of the liquid in the rarefaction region, small vapor filled voids called cavitation bubbles are formed. These cavitation bubbles are formed after a certain threhold of peak negative pressure is applied. This cavitation

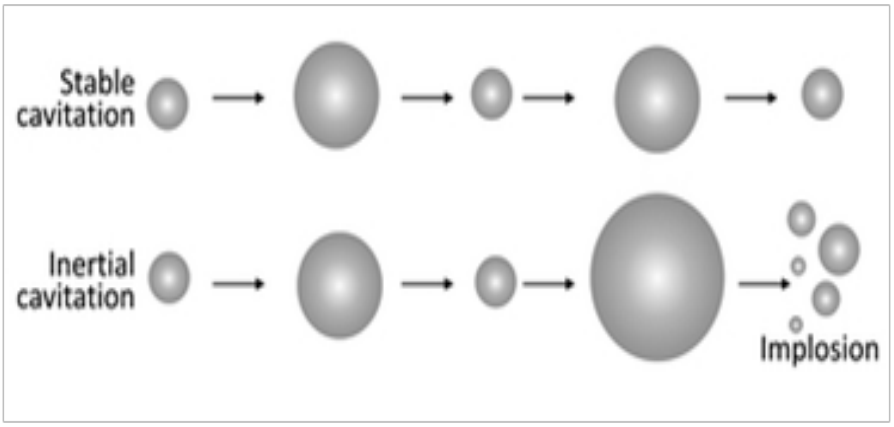

Figure 1-6: Stable and Inertial cavitation.Picture courtesy of [6]

threshold generally increases with increase in frequency of ultrasound [15]. These cavitation bubbles result in microstreaming vortices of sufficient magnitude to result in sonothrombolysis [14] [16]. Microbubbles have been used as a way to lower the cavitation threshold and enhance thrombolysis. To understand the mechanism by which cavitation contributes to sonothrombolysis, numerous studies employed a method known as passive cavitation detection (PCD). In this method an ultrasound transducer is used in receive mode to detect cavitational activity. The Fast fourier transform (FFT) of this detected signal contains spectral information which is then used to analyse the cavitational activity and its effect on sonothrombolysis.

Cavitation is generally classified into two types:

- **Stable cavitation**: In this type of cavitation radial oscillations of the bubble in response to the applied ultrasound takes place and is initiated at lower intensities [16]. Studies have shown ultraharmonic emissions to be the spectral signature associated with stable cavitation and these ultraharmonic emissions have significantly correlated with clot weight loss [17].
- **Inertial cavitation**: In this type of cavitation, rapid increase in size of the bubbles takes place followed by the violent collapse of the bubbles and is initiated at higher intensities/peak negative pressures [16]. Inertial cavitation is characterized by broadband emissions [18][19][17][20]. In a study done by Chen et al (2014) images of microbubble expansion and contraction, culminating in disappearance of the microbbuble and pitted thrombus can be seen. These images show how the mechanical effects of inertial cavitation result in sonothrombolysis.

The role of ultrasound parameters and the type of cavitation that is more effective for different combinations of ultrasound, rtPA and/or microbubbles are not yet completely understood[18]. Further analysis of the mechanisms contributing to sonothrombolysis will be put forth in Chapter 2.

1-5 Proposed solution

The ability of ultrasound to result in thrombolysis forms the basis of proposing a solution wherein ultrasound is used to remove thrombi from the LVAD. This particular problem of pump thrombosis imposes certain restrictions on the delivery of ultrasound to the thrombi found in the LVAD:

Firstly,ultrasound will have to be delivered via a catheter and not a transcutaneous ultrasound transducer as the ultrasound will get attenutated by the metal housing of the pump.

Secondly, the use of rtPA and microbubbles which have shown to enhance thrombolysis will not be utilised. rtPA is associated with co-morbidities and can prove to be more risky than beneficial to the patient [14]. The use of microbubbles will not be effective in a high circulatory device like the LVAD as targetted delivery to the thrombus will not be achieved.

Keeping the aforementioned in mind, the following solution is proposed:

Catheter delivery of ultrasound alone, via the vasculature into the inflow and outflow cannula of the LVAD, with sieves attached to the catheter to collect the emboli released while sonothrombolysis is taking place.

This makes for a therapeutic solution which not only has the potential to remove the thrombi from the LVAD, but also ensure that no further complications are caused by the release of emboli into the blood vessels.

1-6 Research objecives

The main research question of this master thesis is:

Is it possible to remove thrombi from the LVAD using ultrasound?

In order to answer the main research question, sub research questions were formulated.

1. ***What are the ranges of frequencies and intensities of ultrasound which have resulted in a high degree of sonothrombolysis?***
2. ***Which of the following combinations is the most effective for sonothrombolysis?***
    - Ultrasound alone
    - Ultrasound + rtPA
    - Ultrasound + microbubbles

- Ultrasound + microbubbles + rtPA

3. ***What are the mechanisms by which ultrasound alone or in combination with rtPA and/or microbubbles results in sonothrombolysis?***

4. ***For a given frequency of an ultrasound transducer, what is the effect of increasing peak negative pressures on the degree of thrombolysis and at what peak negative pressure is a high degree of sonothrombolysis observed?***

5. ***For a given frequency of an ultrasound transducer, what is the effect of increasing peak negative pressures on cavitational activity?***

1-7 Thesis outline

This thesis is organized as follows:

Chapter 2 is the literature review. In this review the studies that have resulted in a high degree sonothrombolysis have been presented. These studies have been done to investigate the potential of ultrasound in solving potentially fatal consequences of thrombosis ranging from stroke to myocardial infarction. The aim of this literature review was to determine suitable ultrasound parameters required for sonothrombolysis. Since rtpA and microbubbles have been used in the sonothrombolysis studies conducted so far the efficacy of ultrasound in combination with rtPA and or microbubbles, have also been compared. Finally the mechanism via which ultrasound alone or in combination with rtPA and/or microbbubles result in sonothrombolysis have been investigated.

The conclusions from Chapter 2 helped in realising an experimental setup for the preliminary experiments. In Chapter 3, the preliminary experiments conducted have been presented. These preliminary experiments have been conducted to verify conclusions put forth in the literature review. Via PCD the cavitational activity was analysed and a hypothesis regarding the effect of increasing ultrasound intensities on cavitational activity and sonothrombolysis is put forth. An experimental setup to test this hypothesis is realized. Since the aim was to simultaneously try and realise an experimental setup which mimicks the invivo situation the attempts made to do this have been presented. The final experimental setup via which sonothrombolysis and passive cavitation tests will be conducted is presented. The drawbacks with this experimental setup are also explained.

Chapter 4 presents the need for signal conditioning to be able to quantify the raw acoustic signal detected by the PCD. The signal conditioning which will allow for this quantification is presented resulting in the final experimental setup which will be used for the sonothrombolysis and PCD tests on in vitro clots.

Chapter 5 presents the final tests conducted on two sets of clots (6 clots per set). The results of these tests, and conclusions regarding the peak negative pressure (PNP) required

for a high degree of thrombolysis are presented. The experimental evidence regarding the mechanism contributing to a high degree of sonothrombolysis will be presented.

In Chapter 6 the final conclusions based on the experiments conducted in this master thesis are summarized to answer the main research question. The contributions of this thesis work which provide new information to the already existing studies in sonothrombolysis are highlighted. Future work which can be done and which will lead to more insight into the feasibility of the application of ultrasound to remove thrombi from the LVAD is also suggested.

Chapter 2

Review of sonothrombolysis studies

In Chapter 1, the background theory necessary to understand the rest of this master thesis has been presented. In this chapter, the sonothrombolysis studies that have resulted in a high degree of thrombolysis(>50%) have been shortlisted and reviewed. The conclusions from this review will be used to realise an experimental setup that will aid in answering the main research question of this thesis:

Is it possible to remove thrombi from the LVAD pump using ultrasound?

Studies in 2-1 served as the starting point in reviewing the sonothrombolysis studies that have been conducted so far. In this figure the in vivo and invitro studies conducted since 1992 have been presented. These studies have examined the efficacy of ultrasound alone or in combination with microbubbles and/or thrombolytic agents.

From Figure 2-1 we can see that sonothrombolysis was observed for a wide range of frequencies and intensities. The degree of thrombolysis achieved has not been mentioned and for this they will have to be studied individually. From section 1-4 we know that cavitation is one of the dominant mechanisms via which sonothrombolysis takes place. The cavitation threshold increases with increase in insonifying frequency. For a given insonifying frequency stable cavitation occurs at lower ultrasound intensities and inertial cavitation at higher ultrasound intensities. Studies have shown thrombolysis to correlate with both stable and inertial cavitation. For this reason frequency and intensity of ultrasound have a direct effect on the degree of sonothrombolysis.

From this we can conclude that the ultrasound frequency and intensity are crucial parameters when trying to achieve a high degree of sonothrombolysis. In order to obtain a high degree of sonothrombolysis in the experiments conducted towards this master

Reference	Year	PW/CW	US Frequency	W/cm^2	MB	In Vitro/In Vivo	Catheter/ Transcutaneous	Agents	Outcome
38	1999	PW	19.5 kHz	11	Yes	In vitro	Catheter	Heparin, tPA, Tirofiban*	+
39	1999	PW	1.1 mHz	560–2360	Yes	In vitro	Transcutaneous	No	+
37	1999	n/a	33/71 kHz	0.5/3.4	No	In vitro	Transcutaneous	tPA	+
40	1999	PW/CW	10 mHz	0.5–1.0	Yes	In vitro	Catheter	tPA	+
4	1999	n/a	41 kHz	18	No	Human, SVG	Catheter	No	+
31	1999	CW	10.0 mHz	1.02	Yes	In vitro	Catheter	tPA	+
13	1998	PW	37 kHz	n/a	Yes	Rabbit, iliofemoral artery	Transcutaneous	No	+
14	1998	CW	20 kHz/24 kHz	1.5/2.9	Yes	In vitro/rabbit, iliofemoral artery	Transcutaneous	No	+
41	1998	CW	212 kHz/1.0 mHz	0.25/1.0	No	In vitro	Transcutaneous	Urokinase	+
5	1998	PW	1 mHz	1.0	No	Rabbit, retinal vein	Transcutaneous	Streptokinase	+
6	1998	PW	37 kHz	Up to 160	No	Rabbit, iliofemoral artery	Transcutaneous	Streptokinase	+
42	1998	CW	22.5 kHz	30–36	No	In vitro	Catheter	No	+
30	1998	CW	1.0 mHz/20 kHz	420 kPa/ 0.9–5.0 kPa	Yes	In vitro	Transcutaneous	Urokinase	+
43	1997	PW	1.3 mHz	0.3	No	In vitro	Catheter	Urokinase	+
7	1997	Unknown	1.0 mHz	2	No	Rabbit, femoral artery	Transcutaneous	Streptokinase	+
8	1997	PW	19.5 kHz	11	No	Human, coronary	Catheter	Heparin	+
9	1997	n/a	45 kHz	18	No	Human, coronary	Catheter	Heparin/aspirin	+
44	1996	PW	640 kHz	—	No	In vitro	Catheter	Urokinase	+
32	1996	CW	20 kHz	40	Yes	In vitro	Transcutaneous	Urokinase	+
11	1996	CW	26 kHz	18	No	Rabbit, iliofemoral artery	Transcutaneous	Streptokinase	+
29	1995	PW	170 kHz	0.5	Yes	In vitro	Transcutaneous	Urokinase	+
45	1995		1.0 mHz	4	No	In vitro	Transcutaneous	tPA	+
23	1995	PW	0.5, 1.0, 2.3 mHz	0.5	No	In vitro	Transcutaneous	Streptokinase	–
23	1995	PW	0.5, 1.0, 2.3 mHz	0.5–1.5	No	In vitro	Transcutaneous	Streptokinase	+
23	1995	PW	0.5, 1.0, 2.3 mHz	>4	No	In vitro	Transcutaneous	Streptokinase	–
12	1994	CW	1 mHz	2.5	No	In vitro	Transcutaneous	Urokinase	+
46	1994	CW	300 kHz/1.0 mHz	0.07–0.4	No	In vitro	Transcutaneous	tPA	+
22	1994	CW	1.0 mHz	6.3	No	Rabbit, femoral artery	Transcutaneous	tPA, aspirin	–
21	1994	PW	0.17, 1.0 mHz	1	No	In vitro	Transcutaneous	Streptokinase	+
47	1993	CW	20 kHz	1–2	No	In vitro	Catheter	Urokinase	+
19	1993	PW	1.0 mHz	1–2.2	No	In vitro	Transcutaneous	Streptokinase	+
48	1993	CW	0.5 mHz	8.0	No	In vitro	Transcutaneous	tPA	+
49	1992	PW	1.0 mHz	1.75	No	In vitro	Transcutaneous	tPA	+

*Merck, Sharp, & Dohme, White House Station, NJ.
Abbreviations: PW, pulse wave; CW, continuous wave; MB, microbubble; SVG, saphenous vein graft; tPA, tissue-type plasminogen activator.

Figure 2-1: Study done by Porter illustrating the wide range of frequencies and intensities used in sonothrombolysis studies from 1992- 1999

thesis the suitable ultrasound frequency and intensity must be investigated. This led to the formulation of the following research question:

What are the ranges of frequencies and intensities of ultrasound which have resulted in a high degree of sonothrombolysis?

This will be further investigated in section 2-1

From Figure 2-1, we can also see that ultrasound is used in combination with rtPA and/or microbubbles. As explained in section 1-5 the proposed solution to remove thrombi from the LVAD will not employ rtPA and/or microbubbles, i.e ultrasound alone will be applied to remove the thrombi. Hence it is logical to examine the efficacy of ultrasound alone or in combination with rtPA and/or microbubbles. This way an idea of the degree of sonothrombolysis that can or cannot be achieved with ultrasound alone will be known. The degree of thrombolysis achieved for these different combinations will be tabulated and compared to answer the following research question :

Which of the following combinations is the most effective for sonothrombolysis?

- Ultrasound alone
- Ultrasound + rtPA
- Ultrasound + microbubbles
- Ultrasound + microbubbles + rtPA

The detailed study and analysis to answer this research question will be covered in section 2-2.

In the study done by [14] different hypotheses have been put forth regarding the mechanism via which sonothrombolysis takes place. These hypotheses vary depending on whether ultrasound alone is applied or whether ultrasound is used in combination with rtpA and/or microbubbles. The various hypotheses will be studied and analysed with the aim of answering the following research question:

What are the mechanisms via which ultrasound alone, or in combination with rtpa and/or microbbubles results in sonothrombolysis?

This research question will be answered in section 2-3.

2-1 Ultrasound parameters for sonothrombolysis

What are the ranges of frequencies and intensities of ultrasound which have resulted in a high degree of sonothrombolysis?

In order to determine the optimal ultrasound parameters for sonothrombolysis the studies which resulted in a high degree of thrombolysis (DOT) have been analysed. The most commonly used metrics to quantify degree of thrombolysis have been :

- **Clot weight loss (CWL)**: This metric is was employed in sonothrombolysis studies that used in vitro setups. The clot is weighed before and after sonication and the clot loss is calculated.
- **Recanalization (RC)**: Recanalization is the term used to define restoration of blood flow to a vessel which had previously been occluded. The sonothrombolysis studies have used ***Thrombolysis in Myocardial Infarction (TIMI) grade flow scoring system*** to assess the degree of thrombolysis achieved. This is done by visual assessment of the occlusion of the vessel during percutaneous coronary angiography. The TIMI scoring system ranges a score of 0 to 3; with 0 referring to absence of blood flow beyond the occlusion and 3 referring to restoration of normal blood flow to the distal vessels [21].

In the table 2-1 studies that have resulted in a high degree of thrombolysis have been presented. The ultrasound frequency and intensity employed in these studies along with the resulting degree of thrombolysis is tabulated. Further the ultrasound frequency and intensity are classified as shown below:

- Low frequency: 20 kHz to 100 kHz
- Mid frequency: 100 kHz to 1 MHz
- High frequency: 1 MHz and above
- Low intensities: 0.5 W/cm^2 to 10 W/cm^2
- High intensities: >10W/cm^2

From all the studies reviewed the studies that resulted in a high degree of sonothrombolysis (>50%) are shortlisted, these studies are then tabulated with the frequency and intensity that was used for sonothrombolysis. Further the frequency and intensity are classified as mentioned above. This way a conclusion regarding the suitable ultrasound parameters (frequency and intensity) required to obtain a high degree of sonothrombolysis can be arrived at.

In the study done by Steffen (1994) the efficacy of high intensity, low frequency ultrasound for thrombus dissolution in in vitro and in vivo in canine coronary arteries was studied. Ultrasound was applied via catheter at a frequency of 19.5 kHz. The intensity cannot be calculated but we know that a power of 5W is estimated at the catheter tip. When ultrasound was applied with irrigation (i.e infusion of saline) an 80% clot weight loss was recorded. In the invivo studies 13/15 coronary arteries were recanalized in an average of 7 minutes.

In vitro and in vivo tests were conducted in the study done by Ariani (1991). In both the setups ultrasound energy was supplied at a frequency of 20 kHz and the pulsed wave energies varied from 8W to 23 W. The ultrasound intensity cannot be calculated

because of missing information regarding the power at the tip of the catheter. In the in vitro setup 1gram of thrombus was dissolved in 15 seconds or less. The in vivo studies were conducted to thrombolyse 17 acute arterial occlusions in 11 dogs. The thromboses were disrupted in 4 minutes or less. All successful recanalizations were confirmed by angiography.

In the study done by Rosenschein(2000), the safety and efficacy of High intensity focussed ultrasound (HIFU) thrombolysis was studied. In both the in vitro and in vivo studies no microbubbles or thrombolytic agents were used. The relation between ultrasound intensity and thrombolyis efficacy was studied, at intensities $>$ 35 W/cm2 almost complete lysis was achieved.

Ultrasound at an operating frequency of 1MHz and intensity of 20W/cm2 was used with a thrombolytic drug (rtpA) in the study done by Damianou (2013). This study aimed at exploring the application of high intensity and its effect on acceleration of clot lysis. The main results from this paper show that the increase in intensity accelerates the reopening of the carotid artery but it does not stay within the safe temperature limits.

In the study done by Tachibana (1995) albumin microbubbles were used in conjunction with ultrasound. The reasoning was that since cavitation seemed to be the dominant mechanism contributing to sonothrombolyis, using albumin microbubbles to lower the cavitation threhold would accelerate sonothrombolysis. When only urokinase (thrombolytic drug) was used 26.6% was recorded. Ultrasound and urokinase resulted in 33% sonothrombolysis. Further increase in sonothrombolysis - 51%was recorded when ultrasound was used with urokinase and albumin microbubbles.

The aim of the study done by Leeman (2012) was to determine the acoustic requirements for effective microvascular sonothrombolysis. In order to do this an experimental setup was realized, this setup consisted of microthrombi on a pore mesh, pressure was measured upstream of the mesh as an index of thrombus burden. The idea was that when sonothrombolysis would take place this upstream pressure would progressively decrease, thereby serving as a metric for sonothrombolysis. The results of this study showed that more rapid and complete lysis occurred when the ultrasound intensity was increased from 2.4W/cm2 - 137W/cm2.

Table 2-1: Degree of thrombolysis at varying frequencies and intensities

Author	Frequency	Intensity	DOT
Low frequency range ultrasound			
Steffen [22]	19.5kHz	5 W	RC: 13/15 CWL: 80%
Ariani [23]	20kHz	0.6W/cm2	RC:17/17 CWL: 100%
Mid frequency range ultrasound			
Rosenschein[24]	500kHz	40W/cm2	CWL: 90%
Tachibana [25]	170kHz	0.5W/cm2	CWL: 51%
High frequency range ultrasound			
Damianou[26]	1MHz	10-40W/cm2	RC: 22/22
Leeman[18]	1 MHz	2.4-137W/cm2	Near complete lysis

Conclusion

On inspection of the table 2-1, we can conclude that the studies that used high intensities (i.e > 10W/cm2 according to our classification) resulted in a high degree of sonothrombolysis irrespective of the frequency range of the ultrasound.

2-2 Degree of sonothrombolysis for different combinations of US, rtPA and MB

Which of the following combinations is the most effective for sonothrombolysis?

- Ultrasound alone
- Ultrasound + rtPA
- Ultrasound + microbubbles
- Ultrasound + microbubbles + rtPA

The studies will be grouped according to the combinations mentioned above. The idea is to see whether a particular combination results in a higher degree of thrombolysis as compared to the others. This section consists of one table for each combination. To draw a final conclusion as to which combination is most effective the degree of thrombolysis achieved for the different combinations will be compared.

In Table 2-2 the studies done by Rosenschein (2000), Steffen (1994), and Ariani (1991) have been presented. These three studies used ultrasound alone.

Table 2-2: Studies employing ***ultrasound alone*** for thrombolysis

Author	US alone	Degree of thrombolysis
Rosenschein[24]	✓	CWL:90%
Steffen[22]	✓	CWL: 80% RC: 13/15
Ariani[23]	✓	CWL: 100% RC: 17/17

In Table 2-3 the studies done by Eggers (2009), Damianou (2013) and Leeman (2012) have been presented. These three studies used ultrasound + rtpA.

Table 2-3: Studies employing ***ultrasound + rtPA*** for sonothrombolysis

Author	US + rtPA	Degree of thrombolysis
Eggers[27]	✓	CWL: 78.7%
Damianou[26]	✓	RC: 22/22
Leeman[18]	✓	Near complete lysis

In Table 2-4 studies done by Nishioka (1997), Birnbaum (1998) and Leeman (2012) are presented. These studies used ultrasound and microbbubles.

Table 2-4: Studies employing ***ultrasound + MB*** for sonothrombolysis

Author	US + MB	Degree of thrombolysis
Nishioka[28]	✓	RC: 8/11
Birnbaum[29]	✓	RC: 10/10
Leeman[18]	✓	Near complete lysis

Conclusion

On comparing the degree of thrombolysis in table 2-2, 2-3 and 2-4 we can conclude that a high degree of thrombolysis was seen for all combinations of ultrasound and/or rtPA and microbubbles and therefore any one of the four combinations can be employed to achieve a high degree of thrombolysis. However as mentioned in section 1-5 the idea is to use ultrasound alone to remove the thrombi from the LVAD. Given that this analysis shows that ultrasound alone does result in a high degree of thrombolysis we can proceed with realizing an experimental setup using ultrasound alone without thrombolysis enhancing agents like rtPA and microbubbles.

2-3 Mechanisms contributing to sonothrombolysis

What are the mechanisms via which ultrasound alone, or in combination with rtPA and/or microbbubles results in sonothrombolysis?

As discussed earlier acoustic streaming and cavitation are mechanisms contributing to sonothrombolysis. Numerous studies done so far have shown varying degrees of sonothrombolysis partly due to the variations in ultrasound parameters, and choice regarding usage of rtPA and/or microbubbles. Different mechanisms have been hypothesized for ultrasound alone or in combination with rtpA and/or microbubbles. In this section the hypotheses put forth by different studies will be presented. The aim is to gain clarity on the mechanisms contributing to sonothrombolysis for different combinations of ultrasound and/or rtpa and microbubbles.

The aim of the study done by Porter(2001) was to understand the mechanisms contributing to sonothrombolysis. As mentioned in section 1-4 the mechanisms contributing to sonothrombolysis were acoustic streaming and cavitation induced microstreaming. More specifically this study proposed the hypothesis that when ultrasound was used in conjunction with thrombolytic drugs the high velocity gradients caused by streaming increased the exposure of fibrin to the drug, thereby enhancing its effect and resulting in thrombolysis.

In this study, the thermal effect of ultrasound has also been proposed as one the mechanisms contributing to sonothrombolysis however in a study done by Sakharov (2000) the thermal effect has shown to not have a drastic effect on the acceleration of thrombolysis.

In the study done by Xucai chen (2014) the microbubble oscillation and collapse near the surface of the thrombus resulted in a pitted thrombus. This study showed how these mechanical effects could break down the thrombus.

Additonal hypotheses have been put forth by in the study done by Petit (2012), where radial force and microbubbles streaming are hypothesized to contribute to increasing the efficacy of thrombolytic drugs thereby resulting in thrombolysis when ultrasound and thrombolytic drugs are used. The same study hypothesizes that the mechanical effect of cavitation i.e microstreaming in the case of stable cavitation and microjetting in the case of inertial cavitation accelerate clot damage when ultrasound and microbubbles are used.

Conclusion

From the papers reviewed, the following can be stated about the mechanisms by which thrombolysis occurs in different combinations:

- US only: Mechanical cavitation by the bubbles formed from the liquid medium in which ultrasound is propagating.

- Ultrasound + rtPA: When rtPA is used with ultrasound, the ultrasound increases clot permeability enhancing penetration of rtPA into the clot, thus increasing drug efficacy and binding to the fibrin thereby enhancing thrombolysis
- US + MB: Mechanical cavitational effect enhanced by the presence microbubbles (nucleation agent)

2-4 Conclusion

- Low (20 - 100 kHz), middle (100 kHz - 1 MHz) and high (>1 MHz) frequency ranges, all can result in a high degree of thrombolysis provided high intensities (>10 W/cm2) of ultrasound are applied.
- A high degree of thrombolysis was seen for all combinations of ultrasound and/or rtPA and microbubbles and therefore any one of the four combinations can be employed to achieve a high degree of thrombolysis. However as mentioned in section 1-5 the idea is to use ultrasound alone to remove the thrombi from the LVAD. Given that this analysis shows that ultrasound alone does result in a high degree of thrombolysis we can proceed with realizing an experimental setup using ultrasound alone without thrombolysis enhancing agents like rtPA and microbubbles.
- From the studies reviewed conclusions regarding the mechanisms for different combinations of ultrasound and/or rtPA and microbbubles could be drawn. However a clearer understanding of which ultrasound parameters result in cavitation and the mechanism via which cavitation results in a high degree of sonothrombolysis is still unclear. In order to gain clarity on the mechanism contributing to sonothrombolysis, an experimental design and setup wherein the effect of ultrasound parameters on thrombolysis and cavitational activity can be investigated must be realised.

Chapter 3

Design of experimental setup for sonothrombolysis and passive cavitation detection tests

The conclusions drawn from the literature review in section 2-4 have served as the starting point for the realisation of an experimental setup via which preliminary experiments can be conducted.

These preliminary experiments were planned to serve the following purpose:

- to test the conclusions that have been put forth in the literature review regarding optimal ultrasound parameters (frequency and intensity) required for sonothrombolysis
- to build a hypothesis regarding the mechanism contributing to a high degree of sonothrombolysis
- to realize an experimental setup which will mimick the in vivo situation

From the conclusions drawn in section 2-4 regarding the suitable ultrasound parameters (frequency and intensity) required to obtain a high degree of sonothrombolysis, we know that:

Low (20 - 100 kHz), middle (100 kHz - 1 MHz) and high (>1 MHz) frequency ranges, all can result in a high degree of sonothrombolysis provided high intensities (>10 W/cm2) of ultrasound are applied.

Based on this conclusion, the primary/therapeutic ultrasound transducer i.e the ultrasound transducer which is used to to break down the thrombus has to be able to generate high intensity ultrasound waves in order to result in a high degree of sonothrombolysis. Due to a collaboration with University of Cyprus, a (HIFU) transducer with a centre frequency of 1.18MHz was available. Given that it is a high intensity focussed ultrasound transducer the thrombus will have to be aligned to the focus of this HIFU transducer.

As explained in section 1-4 ultrasound in liquid media results in certain phenomena like acoustic streaming and cavitation which have been shown to play a dominant role in contributing to sonothrombolysis. In order to understand the mechanism via which these phenomena contribute to sonothrombolysis a commonly used method known as passive cavitation detection will also be employed in this experimental setup.

The primary reason for understanding any mechanism is to be able to repeat or recreate conditions that will facilitate this mechanism, which in turn, will lead to the desired result. In this particular experiment understanding the mechanism contributing to sonothrombolysis involves understanding how the applied ultrasound parameters result in certain phenomena (acoustic streaming and cavitation), which in turn lead to the desired result of sonothrombolysis. As explained in section 1-4 there are two types of cavitation: stable cavitation occurring at lower ultrasound intensities, and inertial cavitation at higher ultrasound intensities.

Hence the idea of these preliminary experiments is to increase the driving voltage applied to the HIFU, thereby increasing the ultrasound intensities. Via visual observations, the effect of these increasing ultrasound intensities on the clot placed at the focal point of this HIFU will be recorded. In order to understand the mechanism contributing to sonothrombolysis the effect of these increasing ultrasound intensities on cavitational activity(detected via PCD) will also be analysed.

The following investigations will be carried out in two experimental setups:

1. Sonothrombolysis of a clot in a test tube and passive cavitation detection, and
2. Sonothrombolysis of a clot on a rotor and passive cavitation detection.

3-1 Sonothrombolysis of a clot in a test tube and passive cavitation detection

3-1-1 Introduction

This was conducted as a proof of concept in two ways: (i) whether clot formation could take place by following a given clot preparation protocol. The details of the protocol can be referred to in the Appendix A-1, and (ii)whether sonothrombolysis would take place when high intensity ultrasound was applied.

The idea was to increase the driving voltage applied to the HIFU from 100mVpp to 900mVpp in steps of 100mVpp. In preliminary experiments conducted using an ultrasonic cleaner, sonothrombolysis to a degree of 70% was seen when the clot was observed to be *jumping around*. So this was taken to be a visual cue for the onset of high degree of sonothrombolysis. Visual observations regarding the effect of these increasing ultrasound intensities on sonothombolysis will be recorded. At each step of driving voltage applied the cavitation ativity as detected by the PCD will also be observed on the oscilloscope. The detailed description of the instruments and settings used are explained below.

3-1-2 Method and materials

Clot protocol

Fresh porcine blood was taken in a 15ml syringe. This blood was then pipetted into Eppendorf test tubes containing CaCl2 solution (1M) and allowed to coagulate in the same centrifuge tubes.A more detailed description of the clot protocol is given in A-1

In vitro sonothrombolysis setup

As can be seen in Figure the experimental setup employed a function generator (Agilent 33250A), power amplifier (ENI A- 500), oscilloscope (Agilent DS06034A) and a 1.18MHz HIFU. An immersion transducer with a centre frequency of 5MHz was used as the passive cavitation detector (Panametrics V308). The voltage to drive the HIFU transducer, is varied on the function generator. This voltage is varied from 100mVpp - 900mVpp in steps of 100mVpp.

The HIFU transducer has been calibrated in order to determine the peak negative pressures generated by the HIFU transducer for a given range of voltages (100mVpp-900mVpp) applied. The peak negative pressure values (MPa) at the focal point are calculated using the peak negative voltage (mV) recorded by the hydrophone at the focal point and the sensitivity of the hydrophone (38nV/Pa). The graph of this calibration can be referred to in Figure A-2.

The pulsed mode setting is selected on the function generator at a duty cycle of 10% (No of cycles-440, Burst period- 4ms)[30]. This pulsed voltage then gets amplified and is applied to the HIFU transducer which generates pulsed sinusoidal pressure waves that are incident on the blood clot that is placed at the focal point of the HIFU transducer. The focal point of the HIFU was established using the pulser. The detection of the focal point using this pulser can be seen in A-3.

3-1-3 Results

In this experiment the pressure was increased till movement of the clot was observed. Violent movement of the clot was observed once a threshold of peak negative pressure

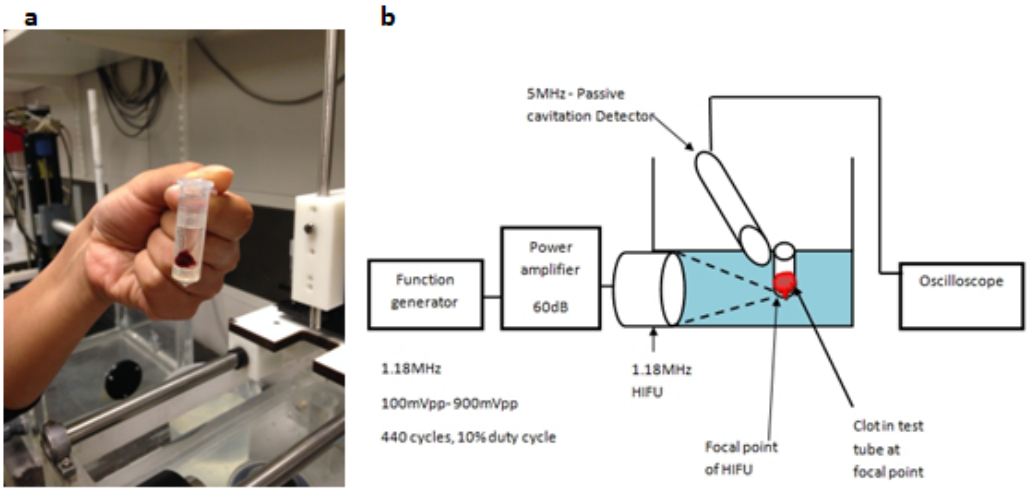

Figure 3-1: a)Clot in saline filled test tube b)Schematic of experimental setup of sonothrombolysis of a clot in a test tube and PCD

was crossed. Minimal movement of the clot was seen at 4.2MPa but no thrombolysis was seen even after 20 minutes. At a pressure of 5MPa violent movement of the clot was seen and in a matter of 15 seconds the clot in the saline solution was reduced to a bloody solution.

Simultaneously, via the method of passive cavitation detection it was observed that intermittent spikes occurred after a certain threshold of driving voltage was crossed. The frequency of occurrence of these intermittent spikes increased, as the driving voltage of the HIFU was increased in steps of 100mVpp.

Hypothesis

The observed relationship between increasing driving voltage of the HIFU, increase in frequency of occurrence of spikes, and resulting sonothrombolysis led to the hypotheses that the increased frequency of occurrence of these spikes contributed to sonothrombolysis. The spikes measured are indicative of the transient collapse of the cavitation bubbles (Li et al., 2014). For the purpose of this experiment they can be termed as ***cavitation events***, initiated by a certain threshold value of driving voltage, which seemed to play a dominant role in thrombolysis.

Trigger threshold and counting cavitation events methodology

The next step after making this observation was to come up with a method to count the number of spikes at each 100mVpp voltage step. This was done by setting a trigger

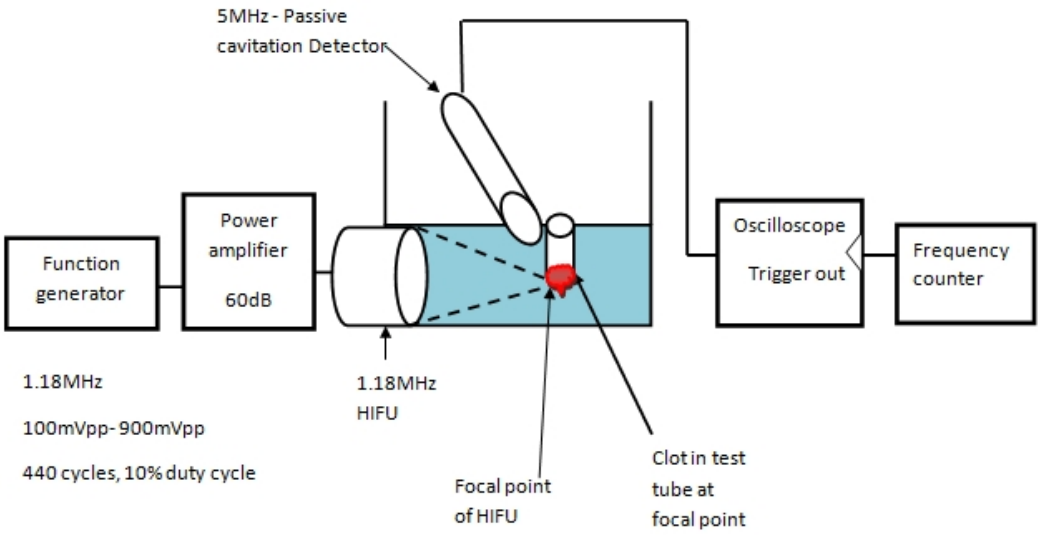

Figure 3-2: Schematic of experimental setup of sonothrombolysis of a clot in a test tube and PCD system with frequency counter

threshold on the oscilloscope. In this experimental setup the oscilloscope serves two purposes:

1. As a PCD signal waveform viewer to view the raw acoustic signals collected by the PCD
2. Measuring device, wherein the feature of trigger threshold is exploited to develop a counting methodology wherein : when the spikes exceed the set trigger threshold a count is triggered, which via, the trigger out feature on the oscilloscope can increment the frequency counter every time this ***cavitation event*** is detected.

3-1-4 Conclusions

A distinct threshold effect was observed between peak negative pressure and sonothrombolysis, beyond which, sonothrombolysis took place in the matter of seconds. The method of passive cavitation detection was employed to understand the role cavitation played in the mechanism contributing to sonothrombolysis. The observations made via the PCD signal seen on the oscilloscope, led to the formulation of a hypothesis and methodology to quantify the cavitational activity detected. The schematic of this experimental setup with the addition of the frequency counter can be seen in Figure 3-2. This method to quantify cavitation and its result on sonothrombolysis will provide experimental evidence, regarding the role these *cavitation events* play in the mechanism contributing to sonothrombolysis.

3-2 Sonothrombolysis of a clot on a rotor and passive cavitation detection

3-2-1 Clot coagulated separately and subsequently placed on a rotor

Introduction

Based on the promising results obtained in the previous experiment, this experiment was planned to see whether the same degree of sonothrombolysis can be achieved when a clot is placed on the rotor and the rotor tip aligned to the focal point of the HIFU. The modus operandi with regards to the design of experiments in this master thesis has been such that each experiment provides answers to questions that will help in objectively assessing whether the proposed solution of using ultrasound to remove thrombi is possible. It is also logical, that, the designs of experiments are planned in context of the situation, in vivo. These in vivo conditions have been elaborated upon in the previous chapters. To briefly reiterate it is a complex hemodynamic environment in which the thrombi are formed on the inlet and sometimes on the surface of the rotor itself.

The research questions looking to be answered via this experiment were:

What is the effect of the polished surface of the rotor on the peak negative pressure required for sonothrombolysis?

How do the *cavitation events* vary with increasing peak negative pressures?

This experiment would give answers regarding the peak negative pressure required for sonothrombolysis of the clot on the surface of the rotor and the passive cavitation detection would help determine whether the same mechanism played a role in sonothrombolysis, when the thrombus was placed on a polished surface like the rotor.

Method and materials

The tip of the rotor was aligned to the focal point of the HIFU transducer. In this experiment a clot was made separately and placed on the rotor. When the clot on the rotor was placed in the water tank at the focal point of the HIFU, the clot floated off the surface of the rotor. Transparent foil was used to hold the clot in place. After removing the transparent foil, when the rotor was placed back into the water tank, the clot did not float off the surface and the experiment was continued.

Results

In this experiment no vibration of the clot on the surface of the rotor was seen at lower peak negative pressures. At a pressure of 3.48MPa the clot was seen vibrating off the surface of the rotor and when this pressure was continuously applied for around 12- 15 minutes the clot peeled off the surface.

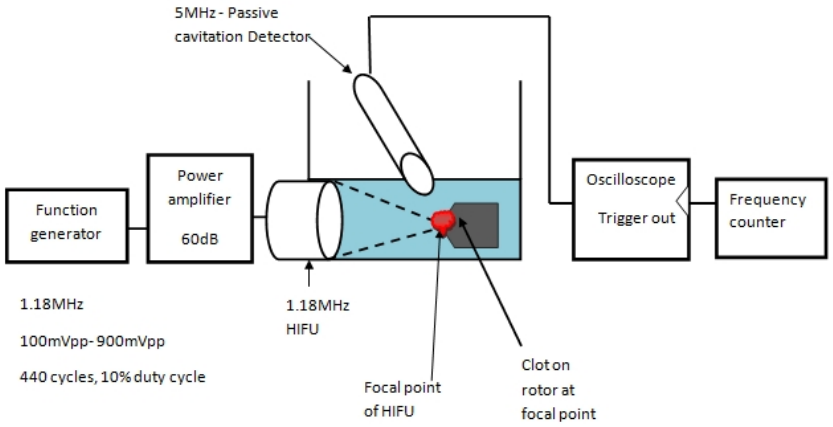

Figure 3-3: Schematic of sonothrombolysis of clot on rotor and PCD

Conclusion

A better way of reproducibly attaching the clot to the surface of the rotor has to be employed if experiments to verify the peak negative pressures required for sonothrombolysis and passive cavitation detection are to be conducted.

3-2-2 Clot coagulated on rotor

Introduction

This experiment is essentially repeating the previous experiment except that the clot is made to coagulate on the rotor in a test tube in an attempt to optimize the way the clot is attached to the surface of the rotor. In Figure 3-4 the rotor placed in the test tube into which porcine blood will be pipetted can be seen.

Method and materials

The clot was left to coagulate in a test tube containing the rotor for 7 days, at the end of this duration the rotor was firmly embedded in the clot in the test tube. As can be seen in Figure 3-4, a grasper was used to grab onto the end of the rotor that is not embedded in the clot in the test tube. A force gauge was connected to this grasper and the force required to dislodge the rotor from the clot in the test tube was measured. This force was measured to be 4.5N. A picture of the clot after dislodging it from the test tube can be seen in 3-5.

The clot was then placed in a holder in the water tank; the drawback inspite of the optimized way of clot formation directly on the rotor method was that blood was found

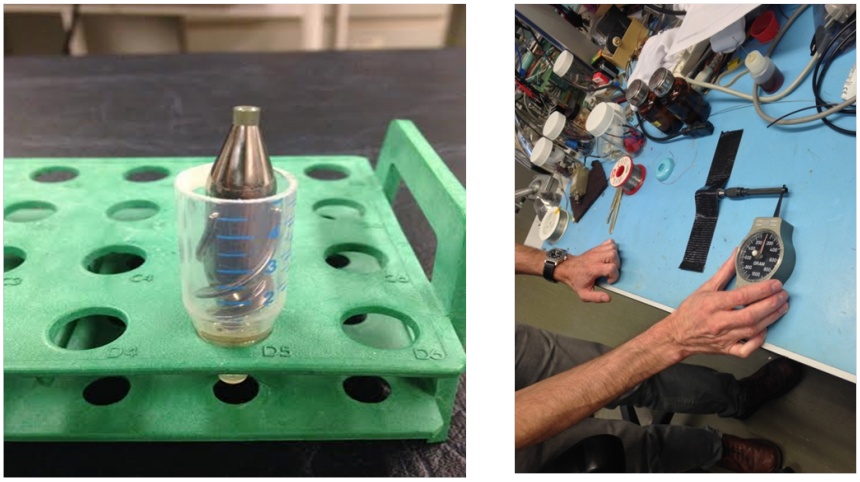

Figure 3-4: Left:Clot cogaulated on rotor, Right:Force gauge to quantify dislodging force of rotor from test tube

to be trickling from the surface of the clot. This would result in an error when it comes to quantifying the weight loss due to sonification alone.

Results

At pressures greater than 2.17MPa the clot was found to be vibrating off the surface of the rotor and peeling off in the order of seconds.

Conclusion

From these two experiments it can be concluded that it is difficult to reproducibly attach the clot to the surface of the rotor. In order to solve the problem of trickling of blood, it was planned to wash and dry the rotor with the clot on it and then perform the experiment. To be sure that the clot was indeed a clot and not dried up blood the clot on the rotor was placed in an egg incubator which maintained a moist environment (prevents drying up of blood) at a temperature of 37 C. Although the clot was attached to the rotor in this experiment, subsequent attempts were unsuccessful.

Hence it was concluded that if the aim was to make invitro clots on the rotor, a future setup would have to include flow of arterial blood over the rotor.

In retrospect, although the experiment of clot on rotor would have made a step towards mimicking the in vivo situation, this experiment ignores the more important effect that

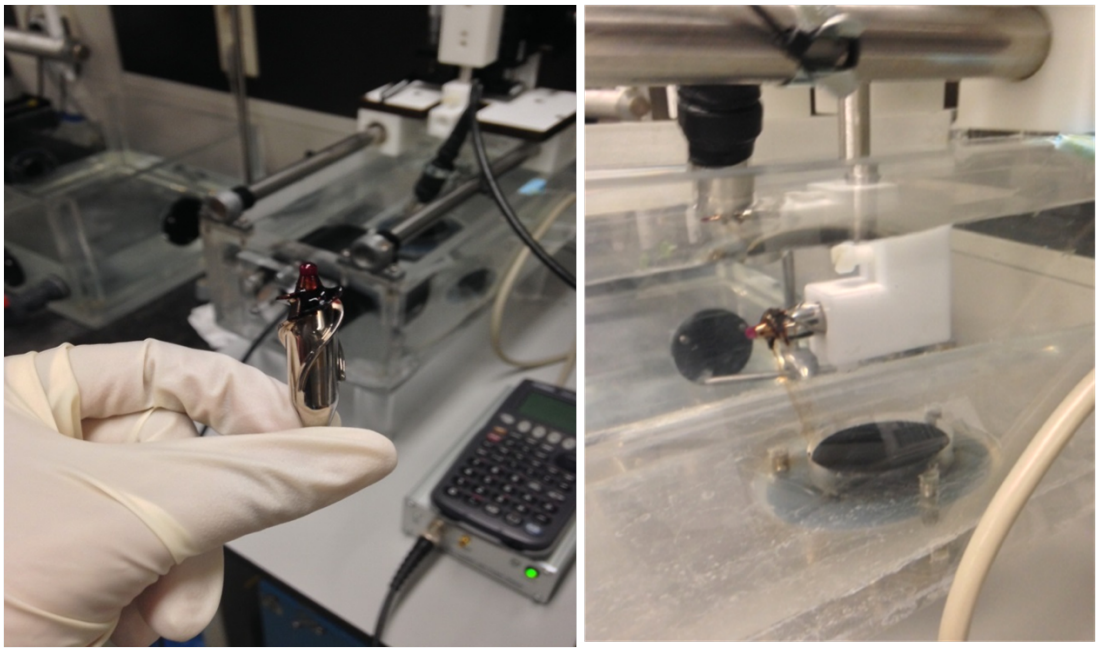

Figure 3-5: Left:Clot on rotor after dislodging from test tube, Right:Blood trickling from rotor when placed in water tank

the housing of the pump will have on the ultrasound field. It is expected that the delivery of ultrasound in to the LVAD via a catheter will undergo reflections, attenuation and scattering. Knowledge of the ultrasound field within the housing of the LVAD will help determine the regions of high and low intensity. Based on this, if high intensity regions are close to the thrombus sonothrombolysis can be expected. If a step has to be made towards mimicking the in vivo situation, it will require modeling of the ultrasound field in the housing of the pump.

3-3 Drawbacks of the experimental setup

From the previous section we know that since a reproducible way of attaching a clot to the surface of the rotor could not be reached, the final experiments that will be conducted in this master thesis will be only:

Sonothrombolysis of a clot in a test tube and passive cavitation detection

These tests were conducted on in vitro clots placed in a saline filled test tube aligned to the focal point of the HIFU.

As mentioned in section the idea of this experiment was to increase the driving voltage of the HIFU and at each step of the driving voltage record the counts or cavitation events

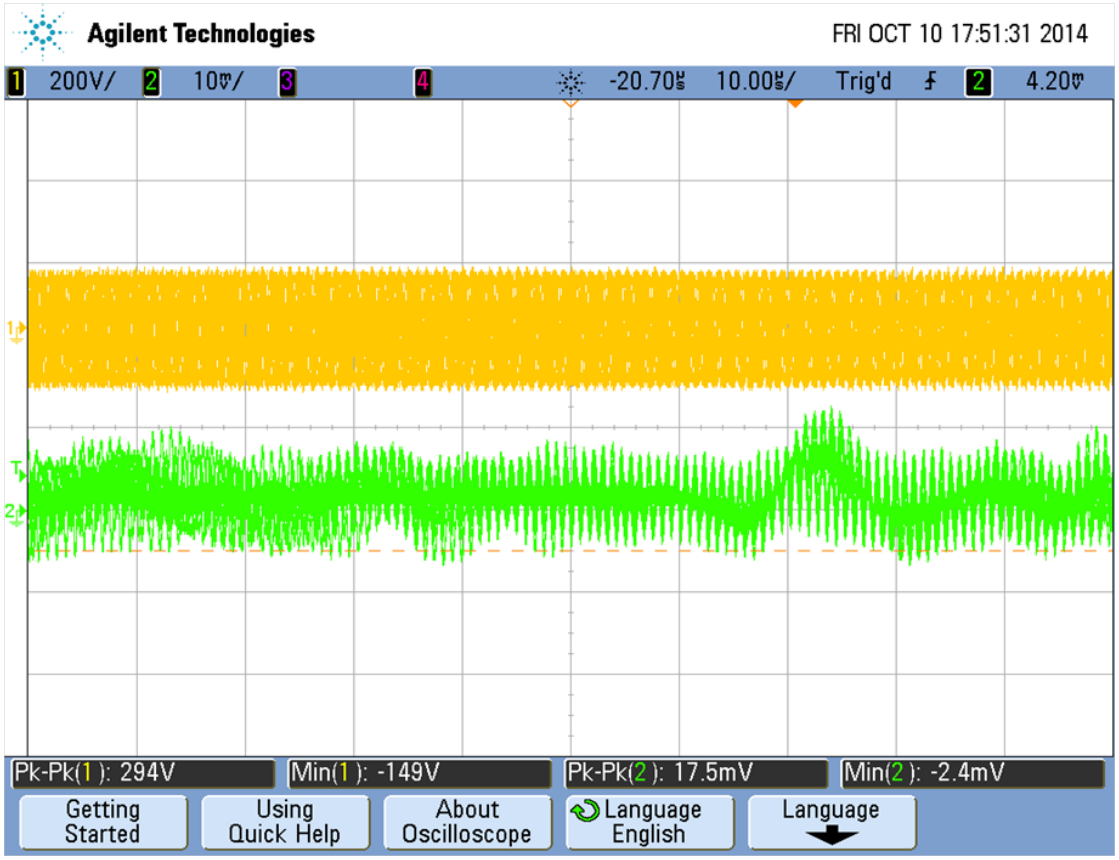

Figure 3-6: Yellow signal: Transmitting HIFU, Green signal: PCD signal

detected via PCD. To reiterate the oscilloscope is used in these experiments to view the PCD signal and trigger a count when a cavitation event is detected. The oscilloscope was setup such that the transmitting HIFU signal could be seen along side the PCD signal.

It was observed that the PCD signal appeared to be a modulated signal, wherein a signal with the same frequency as that of the transmitting HIFU was modulated by a signal with a lower frequency. This can be seen in Figure 3-6.

It was also observed that as the driving voltage was increased the peak to peak amplitude of the PCD signal also increased, this can be seen in the Figure 3-7. This increasing peak peak voltage of the PCD signal made the threshold set redundant. As a solution it was decided that the threshold will also be increased proportionally as the driving voltage was increased, so that the counts could be recorded at each step of driving voltage applied.

However, when this solution was attempted it was observed that the signal was unstable i.e when the clot moved in the test tube the signal moved in tandem, hence making even the *proportionally adjusted threshold* for each driving voltage, redundant. Due to this, the above-mentioned methodology of quantifying cavitational activity by counting the cavitation events could not be employed effectively.

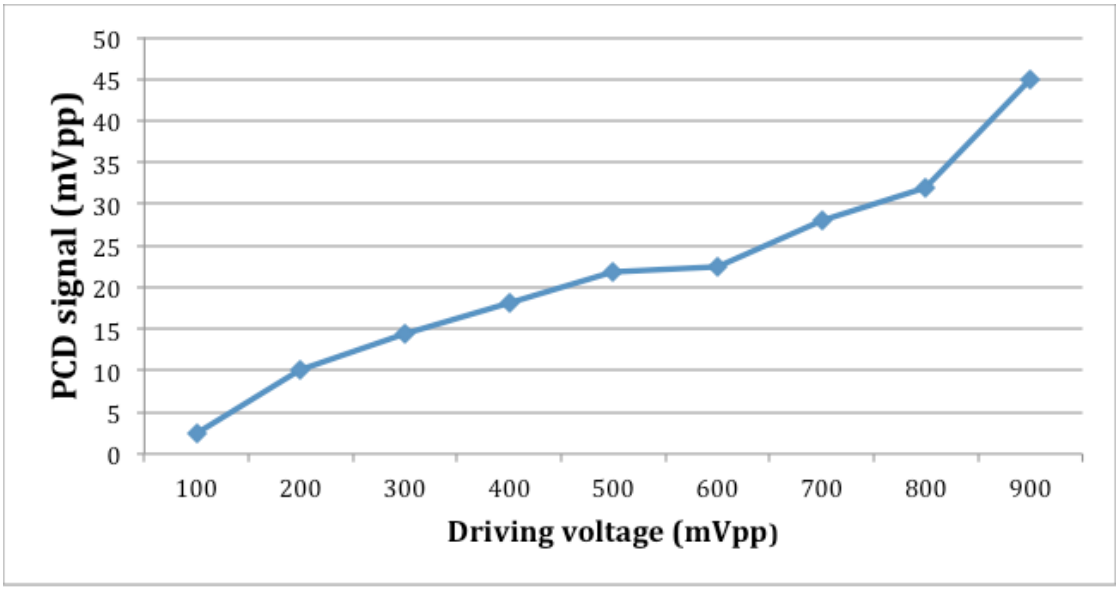

Figure 3-7: Relationship between driving voltage and PCD signal peak peak value

3-4 Conclusion

To reiterate the aim of these preliminary experiments was to:

- to test the conclusions regarding optimal ultrasound parameters (frequency and intensity) required for sonothrombolysis that have been put forth in the literature review
- to build a hypothesis regarding the mechanism contributing to a high degree of sonothrombolysis
- to realize an experimental setup which will mimick the in vivo situation

With regard to testing the frequency and intensity required for sonothrombolysis these preliminary experiments showed that a high degree of sonothrombolysis was observed at high intensities of ultrasound.

Via the method of passive cavitation detection it was observed that the frequency of occurrence of intermittent spikes increased as the ultrasound intensity was increased and it was hypothesized that these spikes termed as cavitation events contributed to sonothrombolysis. A methodology to count these cavitation events with the use of the trigger out feature of the oscilloscope and a frequency counter was realised. This resulted in the realization of an experimental setup wherein the cavitational activity at each step of driving voltage applied could be quantified.

It was the aim to realize an experimental setup which mimicked the in vivo situation. The sonothrombolysis of clot on rotor and passive cavitation detection experiment was an incremental step towards mimicking the invivo situation by attempting to remove the clot on the surface of the rotor. However ,as explained earlier, the methods attempted to attach a clot to the surface of the rotor were not reproducible and hence could not be implemented.

Hence it was decided that the final experiments conducted towards this master thesis would be only: Sonothrombolysis of a clot in a test tube and passive cavitation detection.

However when these tests were conducted it was observed that via this passive cavitation detection system, the counting methodology for quantifying cavitational activity was proving to be unsuccessful. In the next section the frequency content of the PCD signal at each step of driving voltage will be analysed and the solution to this drawback will be presented.

Chapter 4

Signal conditioning for quantification of passive cavitation detection signal

4-1 Frequency content of passive cavitation detection signal

The FFT operation on the oscilloscope was utilised to inspect the frequency content of the raw acoustic signal detected by the PCD. The spectral features of the FFT of this PCD signal were inspected as the driving voltage of the HIFU was increased in steps of 0.1Vpp.

Numerous sonothrombolysis studies done so far have provided experimetal evidence correlating spectral features observed in the PCD signal and resulting sonothrombolysis [18][19][17][20].

On inspection of this FFT, it was observed that after a certain driving voltage was crossed distinct peaks at the fundamental and harmonics could be seen. This is expected at higher intensities due to the nonlinear propagation of ultrasound [31]. This can be seen in Figure 4-1.

It was also observed that a peak emerged between the 3rd and 4th harmonic peaks as the driving voltage was increased and this peak emerged synchronously with the spikes in the time trace of the PCD signal. This can be seen in 4-2

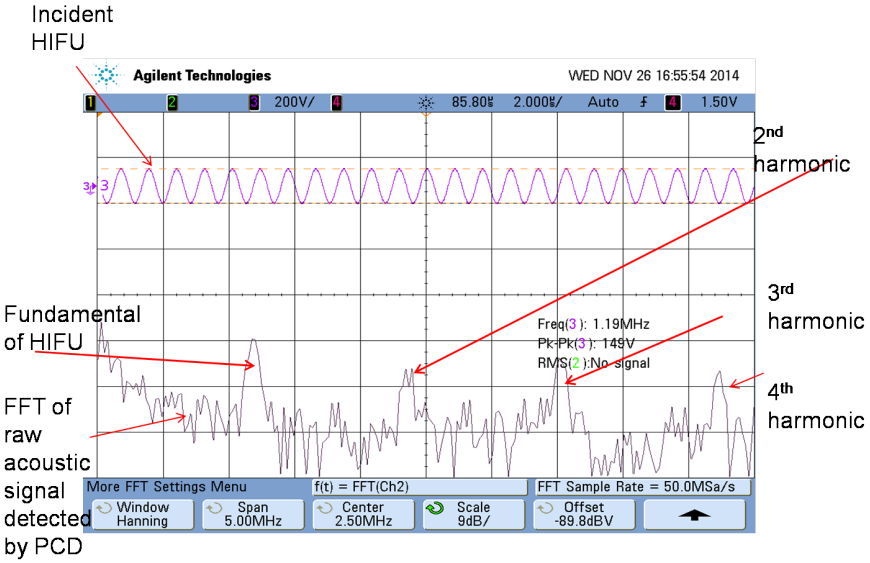

Figure 4-1: FFT of PCD signal at 400mVpp(1.53MPa)

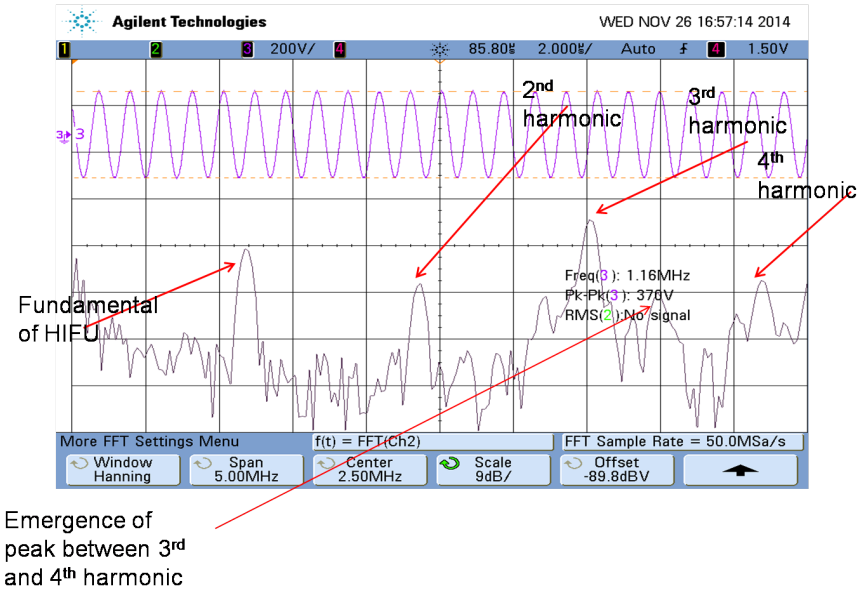

Figure 4-2: Rise in energy level between 3rd and 4th harmonic as driving voltage is increased from 400mVpp(1.53MPa) to 900mVpp(3.18MPa)

4-2 Custom made band pass filter as the solution

In order to extract the signal responsible for the emergence of this peak between the the 3rd and the 4th harmonic, filtering on the raw acoustic signal detected by the PCD will have to be performed. Hence a band pass filter was designed for the frequency band between the 3rd and 4th harmonic, the cut off frequencies were 3.9 and 4.4MHz to ensure that the filtered signal would not contain components of the third and fourth harmonics at 3.54MHz and 4.72MHz. This band pass filter would attenuate the fundamental and its harmonics resulting in a stable signal post filtering. The counting methodology proposed could then be implemented leading to quantification of this filtered cavitation signal.

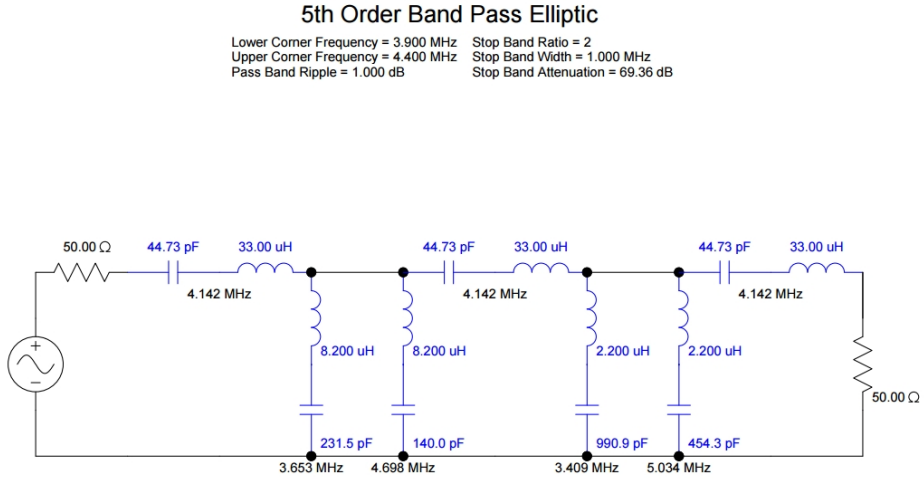

Figure 4-3: Band pass filter circuit

In order to design this filter the software Filter Solution 9.0 by Nuhertz Techonologies LLC was used. The lower corner frequency and the upper corner frequency was input along with the stop band attenuation to obtain the schematic for this band pass filter. The schematic can be seen in Figure 4-3.

4-3 System level design to accomplish quantification of passive cavitation detection signal

The raw acoustic PCD signal will now be filtered and will lead to counting the spikes in this frequency window. The band pass signal is severely attenuated by the filter (60dB) and hence needs to be amplified. The amplifier used is MITEQ, this amplifier is powered by a 15V DC supply. The amplification is done prefiltering so that the noise due to the amplifer can be filtered out. This amplifer has a gain of about 65 dB. In order to avoid distortion the raw acoustic signal detected by the PCD is attenuated and then amplified.

The maximum permissable input to this amplifer is 6mVpp. Clipping of the signal post amplification can be seen in Figure 4-4.

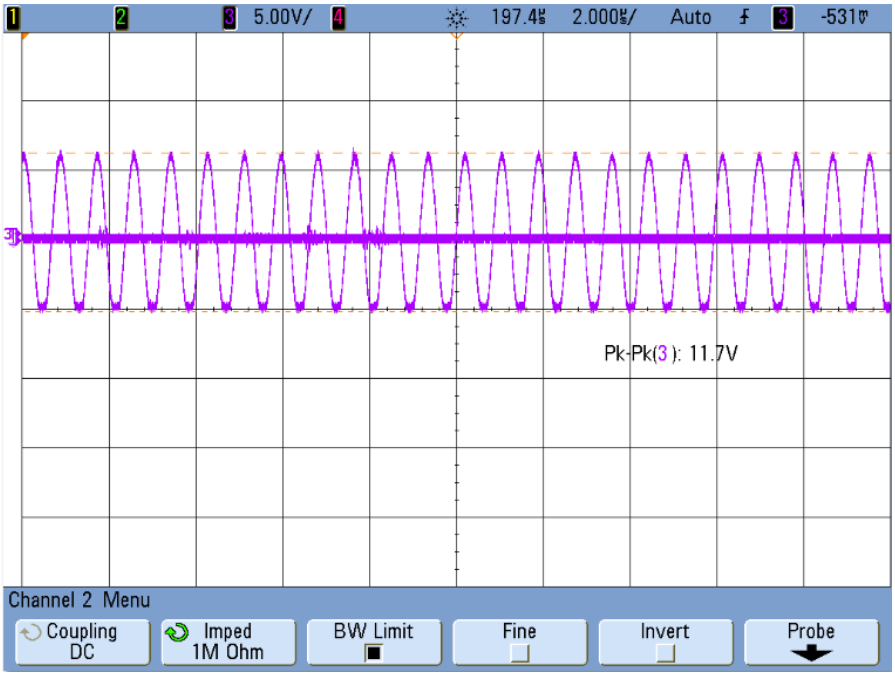

Figure 4-4: Clipping of signal at when input of 7mVpp is given to the amplifier

4-4 Final experimental setup and design

The final experimental setup that will be used to perform the final sonothrombolysis and passive cavitation detection tests can be seen in Figure **??**.

Introduction

In Chapter 3, the experimental design and hypothesis were formulated .In chapter 4, the signal conditioning necessary to quantify the raw acoustic signal detected by the PCD, to test the hypothesis postulated, was realized. The research questions being answered via this setup are:

- What is the Peak negative pressure at which a high degree of sonothrombolysis is observed?
- Does the frequency of occurrence of "cavitation events" increase with increasing peak negative pressures applied and does this increase coincide with a high degree of sonothrombolysis?

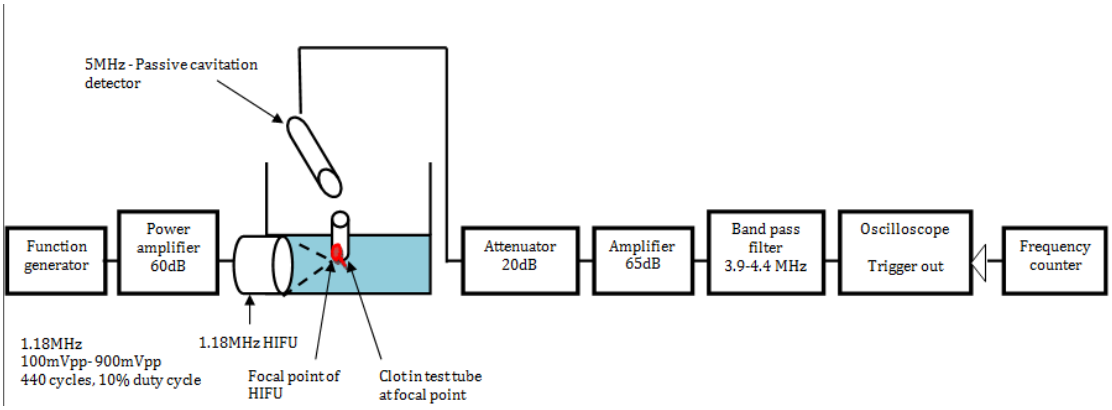

Figure 4-5: Schematic of final experimental setup

Method and materials

As can be seen in the schematic in Figure above, the experimental setup employed a function generator (Agilent 33250A), power amplifier (ENI A- 500), oscilloscope (Agilent DS06034A) and a 1.18MHz HIFU obtained from laboratory collaboration.

The clot is suspended in a test tube filled with saline solution, the test tube is custom made for this experiment with an acoustically transparent plastic window to allow the incident ultrasound waves to reach the blood clot.

The 20 dB attenuator, fixed gain (65dB) amplifier and band pass filter form the signal conditioning block, the trigger out of the oscilloscope is connected to a frequency counter.

As mentioned earlier the driving voltage is increased in steps of 100mVpp, from 100mVpp-900mVpp.

Three observations were made at each step, while conducting these sonothrombolysis and passive cavitation detection tests:

- Counts: When the signal amplitude exceeds the maximum amplitude of the background noise (0.5mV) by a factor 4 (trigger threshold-2mV) a count is triggered. These counts are noted down per minute using a stop watch, at each step of driving voltage applied. This is repeated thrice at each step of driving voltage and the average counts recorded per minute at each step of driving voltage, is calculated. The pulsed incident HIFU along with the filtered PCD signal can be seen in Figure 4-6.
- Color of saline solution: This observation was made to analyze the degree of thrombolysis at different steps of driving voltage applied. Though there are more accurate ways of quantifying the degree of thrombolysis, for example coulter counter. It was not employed as it would not be possible to remove the test tube from the positioning system, pipette out some solution for inspection, and fix it back in the

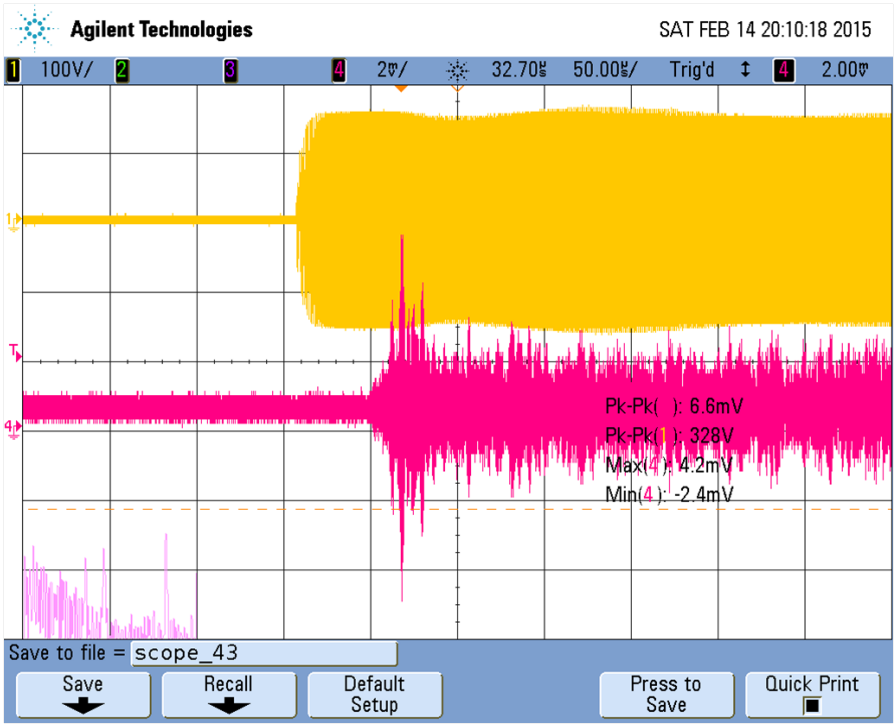

Figure 4-6: Yellow signal: Pulsed incident HIFU, Pink signal : Filtered PCD signal. Background noise of the pink signal when pulsed HIFU is off - 0.5mV, each box of the PCD signal is 2mV.

same position as before. The setup is such that some human error (i.e. affixing test tube to rod) cannot be avoided. The compromise in change of position of the clot in the beam of the incident HIFU was not required especially when it is possible to make reasonably informative observations visually. A completely bloody solution was considered to be an indicator of thrombolysis.

  - Clear saline solution: Indicative of no thrombolysis.
  - Pink saline solution: Indicative of minimal amount of thrombolysis and also the onset of thrombolysis.
  - Red saline solution indicative of substantial amount of thrombolysis. It is important to remember that the solution in the test tube turning red is not indicative of the end of the experiment. The experiment is stopped only in the following situations: (i) When the solution is bloody AND looks completely liquid with no visibly observable particles seen, (ii) When the maximum peak negative pressure of 3.18MPa has been applied.

- Movement: Based on the observations made in the preliminary experiments, movement of the clot at different driving voltages was noted down. The movement of the clot seems to be a function of radial force, acoustical streaming, and cavitation. This observation was made to see the role movement played in the degree of sonothrombolysis achieved. Based on the observations the movement of the clot was classified into the following:

  - Slow: The clot is settled at the bottom of the test tube, slight throbbing motion of the clot without it moving.
  - Swirling: The clot is swirling around the saline filled test tube and is constantly moving
  - Violent: The clot is moving violently at a fixed spot, which can be safely assumed to be the focal point of the HIFU.

If a clot is visible after completion of the experiment, it is weighed again, the degree of sonothrombolysis is calculated (Initial weight minus final weight)/(Initial weight)*100%.

The above mentioned observations will be made during the experiments conducted which are presented in Chapter 5.

The plan for experiments is as follows:

Two sets of 6 invitro clots will be made. Each set of 6 clots will be made from the same batch of porcine blood. The experiment will be conducted on three blood clots on the same day (referred to as *same day clots* below) and the remaining three clots on day 2 (referred to as *one day old clots*). This would shed light on whether there was any correlation between the age of the clot and the peak negative pressure required for sonothrombolysis. This experiment was repeated twice (referred to as set 1 and set 2 below) to verify whether the peak negative pressures required for sonothrombolysis are reproducible.

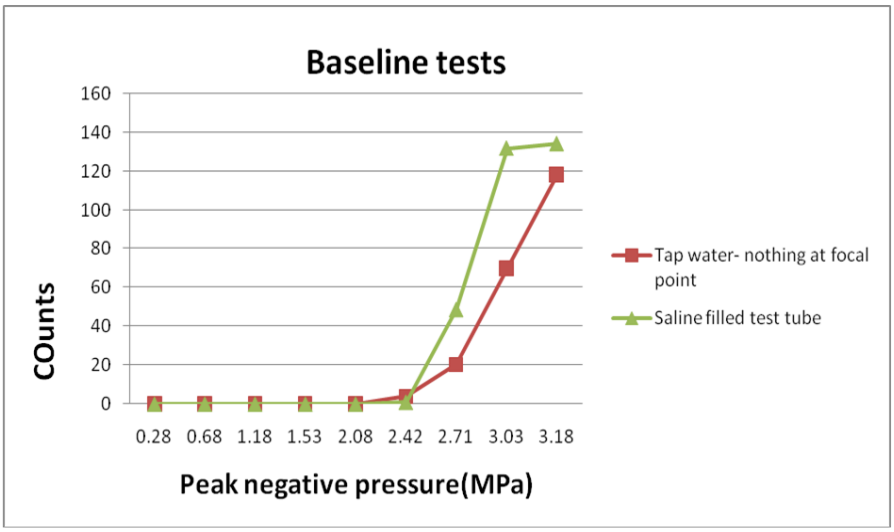

Figure 4-7: Baseline tests conducted to see rate of increase in counts with increasing peak negative pressures

Results

Before the final tests were done on the in vitro clots, baseline tests were performed to see the relationship between peak negative pressure and counts recorded for each step value of driving voltage applied. These baselines tests will enable further inferences to be made regarding how the counts change, when for instance the saline filled test tube is placed, and then, further when the clot is placed in the saline filled test tube.

Baseline tests

The graph in 4-7 is to illustrate the average counts for each peak negative pressure.

- Each data point represents the average counts corresponding to that peak negative pressure.
- The counts are recorded per minute by using a stopwatch. This is repeated three times to calculate the average counts corresponding to that peak negative pressure.

From this graph we can see that the cavitation threshold is the same in both the cases when employing this methodology of counting *cavitation events* to quantify cavitational activity. It is representative of the threshold like behavior which is commonly used to describe cavitation. It can also be inferred that the counts increase slightly more rapidly, i.e. the curve is a little more steeper in the case of the saline filled test tube.

Chapter 5

Final sonothrombolysis and passive cavitation detection tests

5-1 Results of sonothrombolysis and passive cavitation detection tests on Set 1 clots

The graph in 5-1 illustrates the average counts for the range of peak negative pressures applied.

- Each data point represents the average counts corresponding to that peak negative pressure. Each line corresponds to these values per clot.
- The last data point represents the peak negative pressure at which complete thrombolysis was observed (bloody solution by inspection).
- The dotted lines represent the *same day* clots. The solid lines represent the *one-day-old clots*.
- The data points on each of these lines are a particular shape. This shape is colored pink if at that point the saline solution has turned pinkish which indicates minimal amount of thrombolysis. The shape is red if the saline solution has turned red indicative of a substantial amount of thrombolysis.
- This *coloring of data points* is to analyze the counts and degree of thrombolysis with respect to these 6 clots

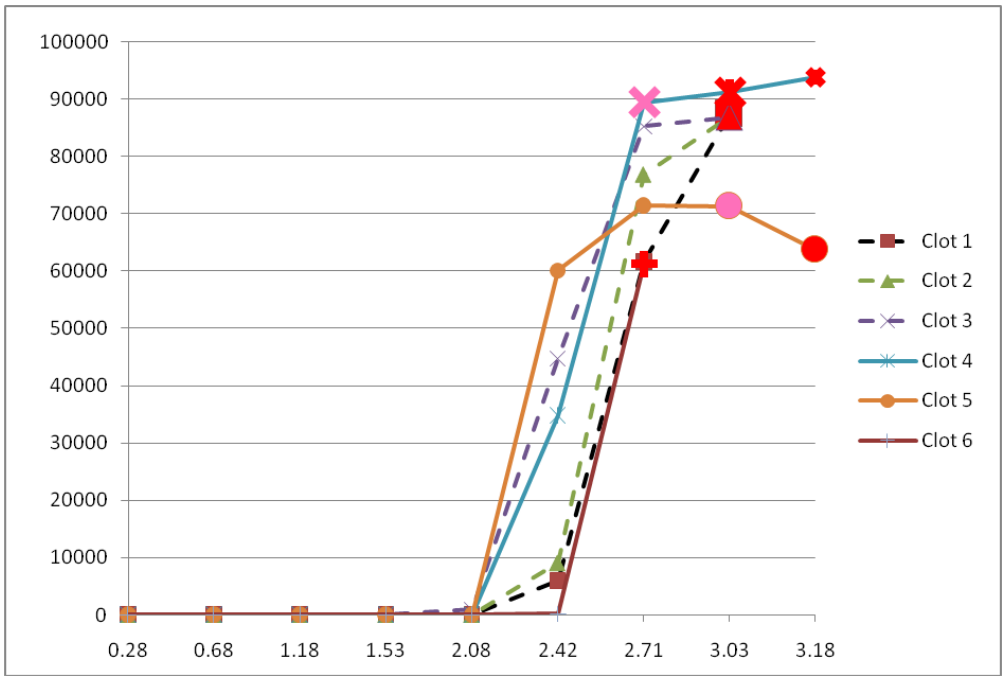

Figure 5-1: Counts measured as peak negative pressures were increased for all 6 clots of set 1

Correlation between clot characteristics and peak negative pressure required for high degree of thrombolysis

Clot weight

To begin with, the clot weight of the invitro clots made cannot be controlled hence the variation in weight between the clots. The invitro clots are left to coagulate in Eppendorf test tubes and while scooping the clot out of the test tube varying weights of clot are obtained. These 6 clots were made using the same clot protocol and each set was made from the same batch of fresh porcine blood obtained. This weight is measured after scooping it out from the Eppendorf test tube. To assess the degree of sonothrombolysis obtained the weight of the clot which remains in the saline filled test tube (if any) is weighed. In Figure 5-2, clot 1 - 6 and their corresponding weights are presented along with the peak negative pressures at which complete sonothrombolysis was observed. It can be seen that no clear correlation is seen between the weight of the clot and the peak negative pressure required for high degree of thrombolysis.

Clot age

From Figure 5-2 clot 1, 2 and 3 (same day clots) all underwent complete thrombolysis at 3.03MPa. Clot 4, 5 (one day clots) underwent thrombolysis at a higher pressure of

	Clot number	Clot weight (mg)	Peak negative pressure(MPa) for high degree of thrombolysis
Set 1	1	43	3.03
	2	30	3.03
	3	28	3.03
	4	22	3.18
	5	79	3.18
	6	29	2.71

Figure 5-2: No correlation observed between clot weight and peak negative pressure required for high degree of thrombolysis

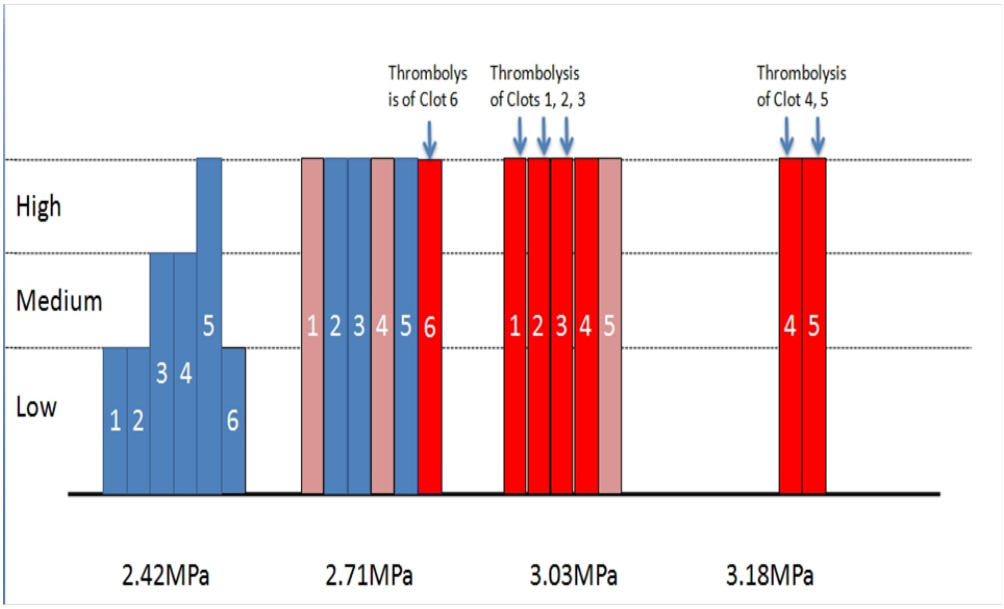

Figure 5-3: Column graph of 6 clots of set 1 giving information of the range of counts detected and degree of thrombolysis observed for each clot at varying peak negative pressure values

3.18MPa. The peak negative pressure required to lyse the clots are all very similar and these results suggest that *same day* clots are easier to lyse than *one day clots*(except clot 6).

Degree of thrombolysis and high counts

The figure 5-3 illustrates the degree of sonothrombolysis for different ranges of counts measured. To reiterate, the counts are measures of the cavitational activity at the focal point of the HIFU, using the trigger threshold counting methodology.

From the Figure 5-3 we can infer the following:

- For 5/6 clots, the onset of high counts is at 2.71MPa

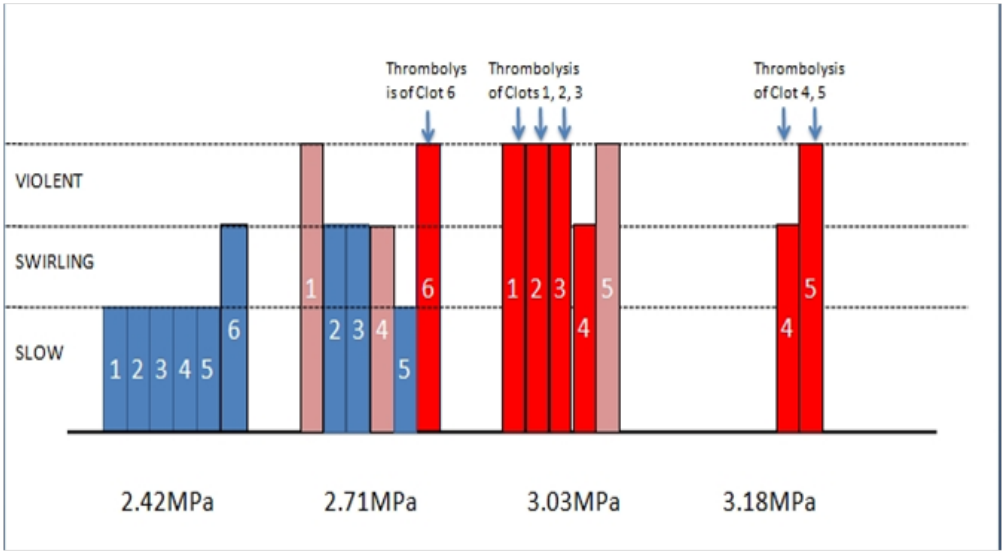

Figure 5-4: Column graph of 6 clots of set 1 depicting the movement of the clot and degree of thrombolysis observed at different peak negative pressures applied

- Rapid thrombolysis clots: Clot 6 rapidly undergoes complete thrombolysis with the onset of high counts. The remaining clots need the driving voltage to be increased to undergo substantial or complete thrombolysis.
- In clot 1 and 4 the high counts do lead to minimal thrombolysis at peak negative pressure of 2.71MPa, but at the next step of driving voltage applied complete thrombolysis takes place
- Clots 1-5 suggest that high counts alone are not sufficient for high degree of thrombolysis.
- Anomaly clots: Clot 5 seems to be an anomaly wherein high counts are seen are recorded at 2.42MPa but a high degree of thrombolysis is observed only at 3.18MPa.

Hence we shall look at the next possible contributing factor to thrombolysis i.e. Movement.

Degree of thrombolysis and movement

The column graph in Figure 5-4 illustrates the movement of the 6 clots of set 1 at peak negative pressures from 2.42MPa to 3.18MPa.

- For 3/6 clots the onset of violent movement is at 3.03MPa.
- In the previous section clots 1-5 did not undergo high degree of thrombolysis despite high counts being measured at 2.71MPa.

- Clots 1-3 underwent high degree of thrombolysis when violent movement accompanied the high counts
- Clot 5 is an anomaly wherein violent movement and high counts led to minimal thrombolysis only
- Clot 4 also seems to be an anomaly wherein no violent movement is observed even at the maximum peak negative pressure of 3.18MPa.
- Anomaly clots: Clot 4 and 5

5-2 Results of sonothrombolysis and passive cavitation detection tests on Set 2 clots

The graph in Figure 5-5 contains information on the average number of counts recorded at each step of driving voltage applied along with the peak negative pressure at which complete thrombolysis was achieved. As explained in section 4-4 the previous experiment is repeated on another set of three same day clots and 3 one day clots to see if the results obtained are reproducible.

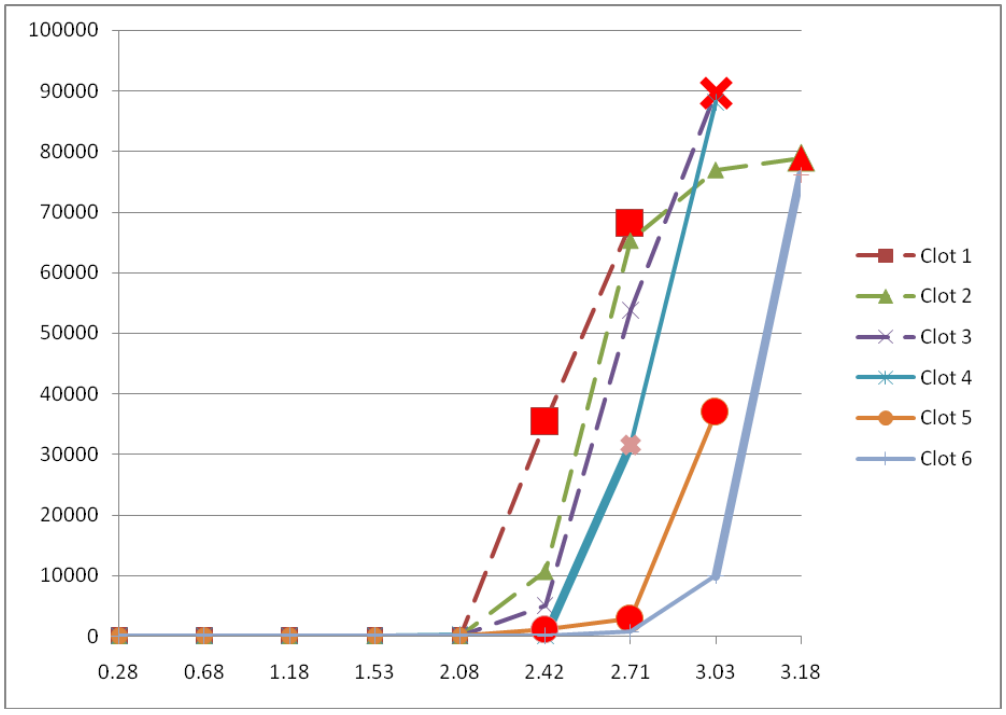

Figure 5-5: Counts measured as peak negative pressures were increased for all 6 clots of set 2

		Clot number	Clot weight (mg)	Peak negative pressure(MPa) for high degree of thrombolysis
Set 2		1	61	2.71
		2	77	3.18
		3	26	3.03
		4	88	3.03
		5	66	3.03
		6	50	3.18

Figure 5-6: No correlation observed between clot weight and peak negative pressure required for a high degree of thrombolysis

Correlation between clot characteristics and peak negative pressure required for high degree of thrombolysis

Clot weight

In Figure 5-6 the six clots of set 2 along with their corresponding weights are presented. Corroborating the conclusion from the previous set, it is seen that no correlation is seen between clot weight and peak negative pressure at which a high degree of thrombolysis was achieved.

Clot age

From Figure 5-6 we can see that clot 1, 2 and 3 (same day clots) undergo complete thrombolysis at different peak negative pressure values 2.71, 3.18 and 3.03 respectively. The one-day clots clot 4, 5 and 6 undergo complete thrombolysis at 3.03, 3.03 and 3.18MPa respectively. The peak negative pressures required for one day clots to undergo complete thrombolysis is slightly higher than same day clots.

Degree of thrombolysis and high counts

In Figure 5-7 the column graph illustrates the counts recorded for the range of peak negative pressures from 2.42MPa to 3.18MPa for 6 clots of set 2.

- For 3/6 clots onset of high counts is at 3.03MPa higher than the previous set which is why the graph also appears wider for the second set.
- Rapid thrombolysis clot: Clot 1, when high counts are recorded complete thrombolysis is observed. Clot 5 undergoes substantial thrombolysis at 2.42MPa itself,

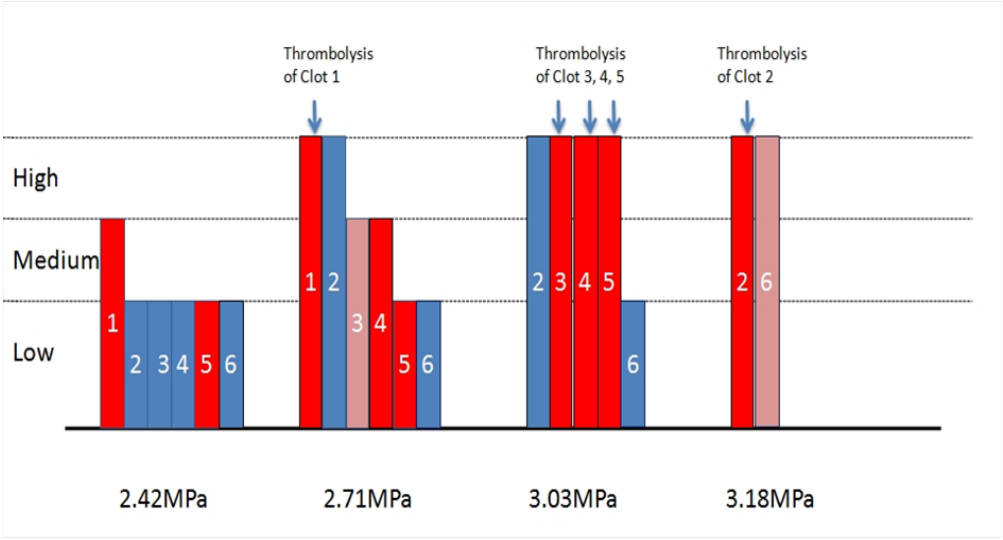

Figure 5-7: Column graph of 6 clots of set 2 giving information of the range of counts detected and degree of thrombolysis observed for each clot at varying peak negative pressure values

but does not undergo complete thrombolysis till 3.03MPa when the counts become high.

- Clots 3 and 4 undergo minimal and substantial thrombolysis at 2.71MPa but are completely gone only when high counts are measured at 3.03MPa.
- Clot 6 is an anomaly where minimal thrombolysis is seen in clot 6 even at the maximum PNP of 3.18MPa. This is probably due to the clot not being at the focal point.

Degree of thrombolysis and movement

The column graph in Figure 5-8 illustrates the movement of the 6 clots of set 2 at peak negative pressures from 2.42MPa to 3.18MPa.

- For 3/6 clots onset of violent movement is at 3.03MPa which is the same as the previous set
- It is observed that clots 3, 4 and 5 undergo complete thrombolysis when accompanied by high counts and violent movement.
- Although the onset of high counts for clot 2 occurs at 3.03MPa, onset of violent movement is only at 3.18MPa and hence complete thrombolysis occurs only then.
- Clot 6 is an anomaly where violent movement does not occur even at 3.18MPa.

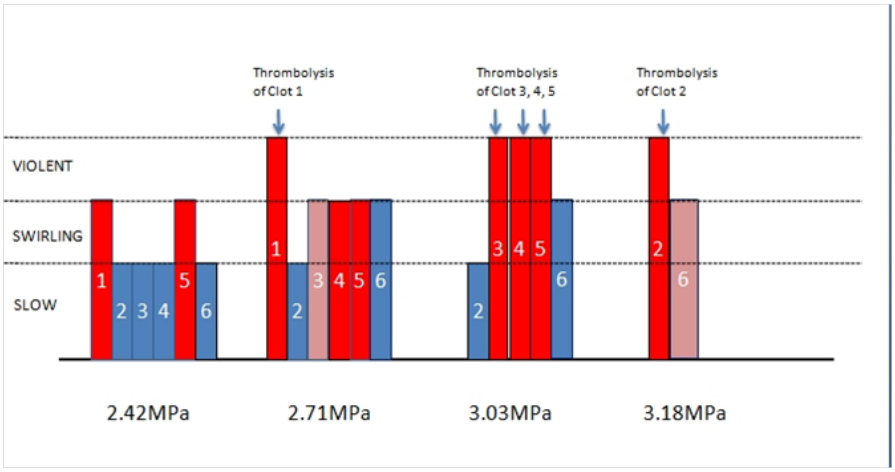

Figure 5-8: Column graph of 6 clots of set 2 depicting the movement of the clot and degree of thrombolysis observed at different peak negative pressures applied

5-3 Condensed results

Figure 5-9 will be explained from left to right, it represents the most important results from the experiments conducted and has been compiled by condensing the tabulated results of each of the 12 clots. Refer appendix 1 The main results to be taken away from this tabular column are the following:

- No clear correlation exists between clot weight and peak negative pressure at which high degree or complete thrombolysis was observed. A slightly higher peak negative pressure was required for one day clots to undergo complete sonothrombolysis.
- The peak negative pressure at which complete thrombolysis was first observed was 2.71MPa (2/12 clots), however majority (10/12) of the clots required higher peak negative pressures of 3.03MPa and 3.18MPa before achieving complete or significant amount of thrombolysis.
- Clots with significant thrombolysis (11/12) were noted to have high counts (11/11 clots) and violent movement (10/11)
- Clot 6 in the second set was the only clot where significant degree of thrombolysis was not achieved despite having high counts and violent movement. The only possible explanation is that the clot may not have been at the focal point of the HIFU.

	Clot number	Clot weight (mg)	Clot age (hours)	Peak negative pressure(MPa)	Degree of thrombolysis	Movement	Count
	1	43	5	3.03	100%	Violent	High
	2	30	6	3.03	100%	Violent	High
Set 1	3	28	7	3.03	100%	Violent	High
	4	22	24	3.18	86%	Swirling	High
	5	79	25	3.18	100%	Violent	High
	6	29	26	2.71	93%	Violent	High
	1	61	5	2.71	100%	Violent	High
	2	77	6	3.18	89%	Violent	High
	3	26	7	3.03	100%	Violent	High
Set 2							
	4	88	24	3.03	100%	Violent	High
	5	66	25	3.03	97%	Violent	High
	6	50	26	3.18	32%	Violent	High

Figure 5-9: Condensed results of Set 1 and Set 2 illustrating that high degree of thrombolysis was accompanied with high counts and violent movement

5-4 Discussion

In this section, the results of this work are discussed in relation to the other sonothrombolysis studies done.

How much information does this counting methodology really give us about the cavitation events that lead to thrombolysis?

In this study we are counting the spikes observed in the time trace as the driving voltage/peak negative pressure is increased. Firstly, we will discuss the source or the physical effects which result in these spikes. When cavitation bubbles oscillate rapidly and collapse several physical effects are generated namely shockwaves, micro jets, turbulence, shear forces etc. In the study done by Li (2014) the inertial cavitation bubble collapse is modelled by a unipolar spike.

As mentioned in section inertial cavitation is characterized by broadband emissions. Since the spikes that are being counted in this experiment are broadband, we assume that they are the result of the physical effects characteristic to inertial cavitation. Keeping this in mind, we can say that the counts recorded at each step of driving voltage serves as a metric of inertial cavitation.

In the results of this study we see that these counts increase after a certain threshold of peak negative pressure is crossed and in majority of the clots high counts are recorded

at 2.71MPa. However, in these results we also see that high counts do not always lead to a high degree of thrombolysis. This suggests that these physical effects that lead to high counts at lower peak negative pressures are less powerful than those at higher peak negative pressures.

Are observations linking cavitation, movement and thrombolysis insightful?

In this study the movement of the clot and the degree of thrombolysis for peak negative pressures from 2.42MPa - 3.18MPa have been presented.

In all the clots, violent movement has been accompanied by high counts, i.e violent movement has always been accompanied by inertial cavitation and resulted in a high degree of sonothrombolysis. That being said a high degree of thrombolysis was not achieved with violent movement alone. This was verified by vigorously shaking the saline filled test tube containing the clot. This served as a falsifying experiment confirming that violent movement alone cannot result in a high degree of sonothrombolysis. This underlines the importance of inertial cavitation in achieving a high degree of sonothrombolysis. Hence it has been insightful to record observations linking cavitation, movement and thrombolysis as it helped ascertain the dominant mechanism contributing to sonothrombolysis.

5-5 Conclusion

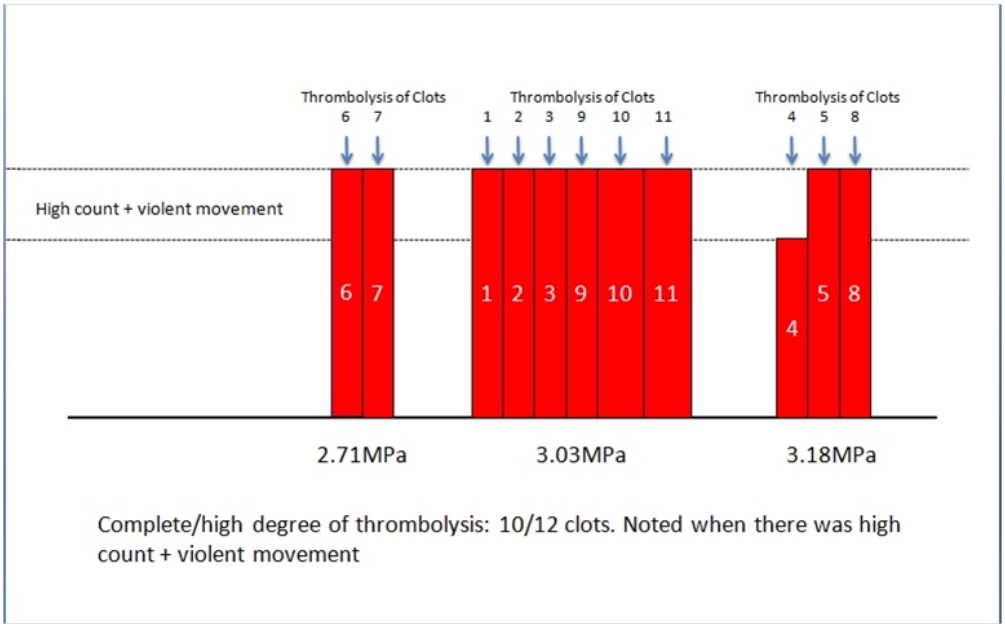

Figure 5-10: High degree of thrombolysis was seen in majority of the clots all of which were accompanied by violent movement and high counts

From Figure 5-10 it is evident that for majority of the clots, thrombolysis occurs at a peak negative pressure of 2.71MPa - 3.18 MPa. Clots which underwent a high degree of sonothrombolysis were always accompanied by high counts and violent movement. We assume the intermittent spikes termed as *cavitation events* being counted are due to physical effects like inertial bubble collapse, shockwaves and microjets, which are characteristic of inertial cavitation. Hence we can conclude that the high counts are indicative of inertial cavitation and play a dominant role in achieving a high degree of sonothrombolysis.

Chapter 6

Conclusions and Recommendations

6-1 Summary and conclusion

Longer life expectancy coupled with the information that cardiac heart failure is on the rise, are strong indications that the LVAD will be a crucial device in the time to come. Not only does this device sustain and extend life, it provides a quality of life which would not have been possible for patients suffering from end stage heart failure.

One of the main drawbacks plaguing this otherwise successful medical device is the formation of thrombi. The formation of these thrombi on the inlet bearing and/or on the rotor of the LVAD occludes the blood flow and prevents it from functioning properly. The aim therefore is to remove the thrombi, thereby restoring unoccluded blood flow in the LVAD. The potential of ultrasound to remove thrombi was first reported by Trubestein in 1976. In the next 25 years numerous sonothrombolysis studies have corroborated these results and used ultrasound to enhance the effect of thrombolytic agents [14]. In the studies done in more recent times experimental evidence elucidating the effects of ultrasound in liquid media and how these effects result in a high degree of sonothrombolysis has been put forth [32] [20]. In light of the promising results of the sonothrombolysis studies done so far ultrasound was proposed as the ***means*** to achieve the aim of removing thrombi and restoring blood flow through the LVAD.

To explain the proposed solution more specifically, the idea is: catheter delivery of ultrasound alone, via the vasculature into the inflow and outflow cannula of the LVAD, with sieves attached to the catheter to collect the emboli released while sonothrombolysis is taking place.

This makes for a therapeutic solution which not only has the potential to remove the thrombi from the LVAD, but also ensure that no further complications are caused by

the release of emboli into the blood vessels. The promising results of sonothrombolysis seen thus far, coupled with the recent development of emboli protective devices make this solution less fantastical and more probable.

The first step towards answering the main research question was to review the sonothrombolysis studies done so far. From this review it was concluded that for a high degree of thrombolysis to take place an ultrasound transducer of any range of frequency can be used provided that high intensity is applied. Good thrombolysis efficacy was seen for all combinations of ultrasound and/or rtPA and microbubbles. Although several studies provided experimental evidence regarding the mechanism via which sonothrombolysis took place, the effect of increasing ultrasound intensities on cavitational activity and whether stable or inertial cavitation is more effective is not completely understood.

The conclusions from the literature review helped realize an experimental setup. This experimental setup consisted of a high intensity focussed ultrasound transducers which would generate mechanical pressure waves to break down the clot and a passive cavitation detector to analyze cavitational activity. Preliminary experiments conducted verified that high intensity ultrasound could result in a high degree of sonothrombolysis. Via PCD it was observed that increasing ultrasound intensities resulted in an increase in the frequency of occurrence of intermittent spikes. Based on this observation it was hypothesized that the increased frequency of occurrence of spikes contributed to a high degree of sonothrombolysis. A methodology to count these spikes at each step of driving voltage applied was implemented by using the trigger out feature of the oscilloscope and a frequency counter. In order to count these spikes, signal conditioning had to be performed. The frequency content of the PCD signal was analyzed and it was found that filtering the PCD signal for the band between 3rd and 4th harmonics would result in a time domain signal representative of inertial cavitation. Since the filter attenuated the PCD, signal amplification was performed using an off the shelf amplifier which was powered by a 15V DC supply. However to prevent distortion, the PCD signal had to be attenuated such that it was the within the permissible input levels of this amplifier. Post this signal conditioning, a passive cavitation detection system was realized via which the intermittent spikes in the PCD signal could be counted. Finally, an experimental setup to conduct sonothrombolysis and passive cavitation detection tests was realised.

Via this experimental setup the first strides towards exploring this proposed solution have been made. In the final experiment sonothrombolysis and passive cavitation detection tests are conducted on two sets of 6 clots each. This experiment was conducted to answer the following questions:

- What is the Peak negative pressure at which a high degree of thrombolysis is observed?
- Does the frequency of occurrence of "cavitation events" increase with increasing peak negative pressures applied and does this increase coincide with a high degree of thrombolysis?

On analysis of the results of the final sonothrombolysis and passive cavitation detection tests the following conclusions were drawn:

For majority of the clots, sonothrombolysis occurs at a peak negative pressures of 2.71MPa - 3.18 MPa. We assume the intermittent spikes termed as *cavitation events* being counted are due to physical effects like inertial bubble collapse, shockwaves and microjets which are characteristic of inertial cavitation. Hence we can conclude that the high counts are indicative of inertial cavitation and play a dominant role in achieving a high degree of sonothrombolysis. That being said high counts did not always result in a high degree of sonothrombolysis and this is probably because the physical effects that occur at lower peak negative pressures are less powerful than those at higher peak negative pressures. From this we can conclude that the peak negative pressure is a good indicator of whether a high degree of sonothrombolysis would take place. This means that the peak negative pressure at which sonothrombolysis take place can be used in the next step of catheter development. The ultrasound transducer of the catheter must be able to generate a certain threshold of peak negative pressure in order to achieve a high degree of sonothrombolysis.

To answer the main research question of this thesis:

Is it possible to remove thrombi from the LVAD pump using ultrasound?

The results of the experiments conducted towards this master thesis are very promising. A high degree of thrombolysis was observed in 11/12 clots. Experimental evidence elucidating the role of inertial cavitation in achieving a high degree of sonothrombolysis and why a certain threshold of peak negative pressure must be attained in order to achieve a high degree of sonothrombolysis has been put forth. This evidence can be utilized in the next step of catheter design. At this stage it can be said that the application of ultrasound to remove thrombi from the LVAD will prove to be successful if high intensity ultrasound resulting in inertial cavitation can be delivered to the site of the thrombi formation in the LVAD.

6-2 Contribution

- Literature review - From chapter 2 it is clear that there were a wide range of frequencies and intensities used in sonothrombolysis studies. An important contribution of this thesis are the conclusions from the literature review which showed that high degree of thrombolysis could be achieved at any frequency range provided high intensities were used.
- Via the preliminary experiments, the challenges surrounding attaching clots to the surface of the rotor have been highlighted and this information is relevant for studies attempting to reproducibly attach clots onto a surface.

- Based on the observations made in preliminary experiments a methodology to quantify cavitation activity at different ultrasound intensities was realized. This quantification involved counting intermittent spikes at each step of driving voltage applied. On analysis of the frequency content of this signal it was observed that these spikes were broadband, hence it was assumed that they are the result of the physical effects characteristic to inertial cavitation. This way the counts at each step of driving voltage applied served as a metric of inertial cavitation. The necessary signal conditioning to realize this quantification was implemented resulting in an experimental setup which could answer questions regarding the effect of increasing peak negative pressures on cavitational activity and thrombolysis.
- Most importantly experimental evidence regarding the need for a certain value of peak negative pressure to observe a high degree of sonothrombolysis has been presented.

6-3 Future recommendations

- Clot characterization - A study done by Sutton (2013) it was concluded that the clot composition and structure could inhibit the sonothrombolysis achieved [33]. This has not been looked into in this study. In order to investigate this sonothrombolysis tests will have to be conducted on thrombi retrieved from explanted LVADs.
- Catheter development - The conclusions of this master thesis emphasize the need for a certain threshold value of peak negative pressure necessary for a high degree of sonothrombolysis. The proposed solution entails intravascular delivery of ultrasound. The catheter can consists of a piezoelectric transducer or a CMUT. Capacitive micromachined ultrasound transducers have shown to have advantages over piezoelectric transducers for HIFU applications. Simulations from a study done on design for a CMUT ablation catheter show that it can only produce 800kPa. If the aim is to implement a CMUT catheter for this application of removing thrombi from the LVAD , then experiments using a lower insonifying frequency can be attempted. The cavitation threhold decreases with decrease in insonifying frequency which might lead to a lower peak negative pressure required for sonothrombolysis.
- Modelling of the LVAD pump - It is expected that the ultrasound delivered via the catheter into the LVAD will undergo reflections, attenuation and scattering. The reflections will lead to the formation of standing waves. It is known that these standing waves impact regions of high and low intensities. The results of this thesis, underline the importance of inertial cavitation in achieving a high degree of thrombolysis, and the dependence of this inertial cavitation on high intensity ultrasound, hence the modelling of the pump is very crucial to determine regions of high and low intensity. The aim being that the high intensity regions are close to the thrombus surface.

Appendix A

Appendix A

A-1 Clot protocol

Old Protocol

The clot protocol used for the initial set of experiments is explained below: The clots were produced using blood stored in 4.5ml tubes(BD Vacutainer, US) containing 0.5ml citrate solution(0.105M), which prevents the blood from coagulating and was kept in the refrigerator. Whenever a clot was required to be made, the frozen blood which was aliquoted into centrifuge tubes was taken out and left to thaw for approximately thirty minutes. After the blood was thawed,750ÂţL of the blood was mixed with 60ÂţL of CaCl2 solution. The CaCl2 solution was prepared mixing deionized water with granular anhydrous CaCl2 The tubes containing the mixture of blood and CaCl2 were then incubated at room temperature for 3hours. This clot protocol led to problems in the formation of the clot, the preparation was gone through in detail. These problems could have risen due to the freezing and thawing and improper mixing which might destroy blood cells. Blood is delicate and rough handling while mixing can destroy the cells.

New protocol

This considered a new protocol was formulated wherein fresh blood from a pig was taken in a 15ml syringe. This blood was then pipetted into Eppendorf test tubes containing CaCl2 solution(1M) and allowed to coagulate in the same centrifuge tubes. It is important to note that in this case it is used to accelerate coagulation, previously it was used to compensate for the already added anticoagulant. The syringe and the valve system used to pipette the blood into the eppendorf testtubes can be seen in A-1

Eppendorf tubes with 38 µL $CaCl_2$ coagulating solution already pipetted

15 ml syringe, valve connector and 1 ml syringe to pipette 750µL fresh porcine blood into eppendorf tubes

Figure A-1: Clot preparation protocol

A-2 HIFU calibration

The HIFU transducer has been calibrated in order to determine the peak negative pressures generated by the HIFU transducer for a given range of voltages (100mVpp-900mVpp) applied. The pressure values (MPa) at the focal point are calculated using the peak negative voltage (mV) recorded by the hydrophone at the focal point and the sensitivity of the hydrophone (38nV/Pa). This can be seen in A-2.

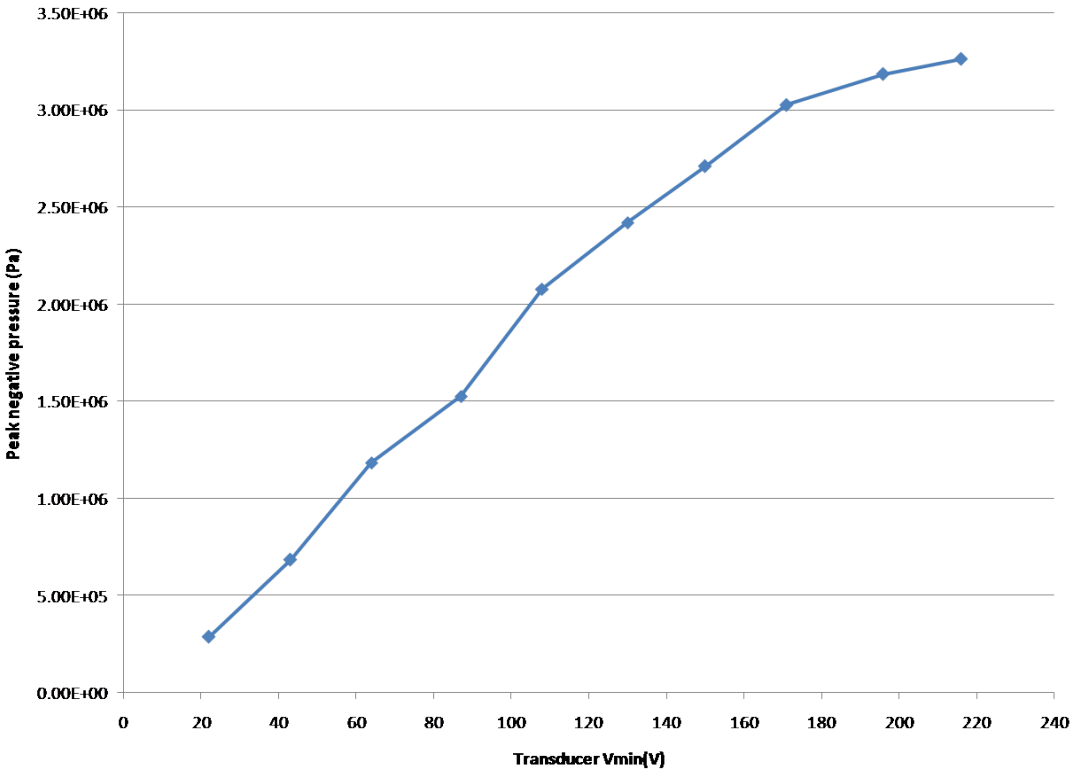

Figure A-2: Calibration of the HIFU

A-3 Locating focal point of HIFU using pulsar receiver

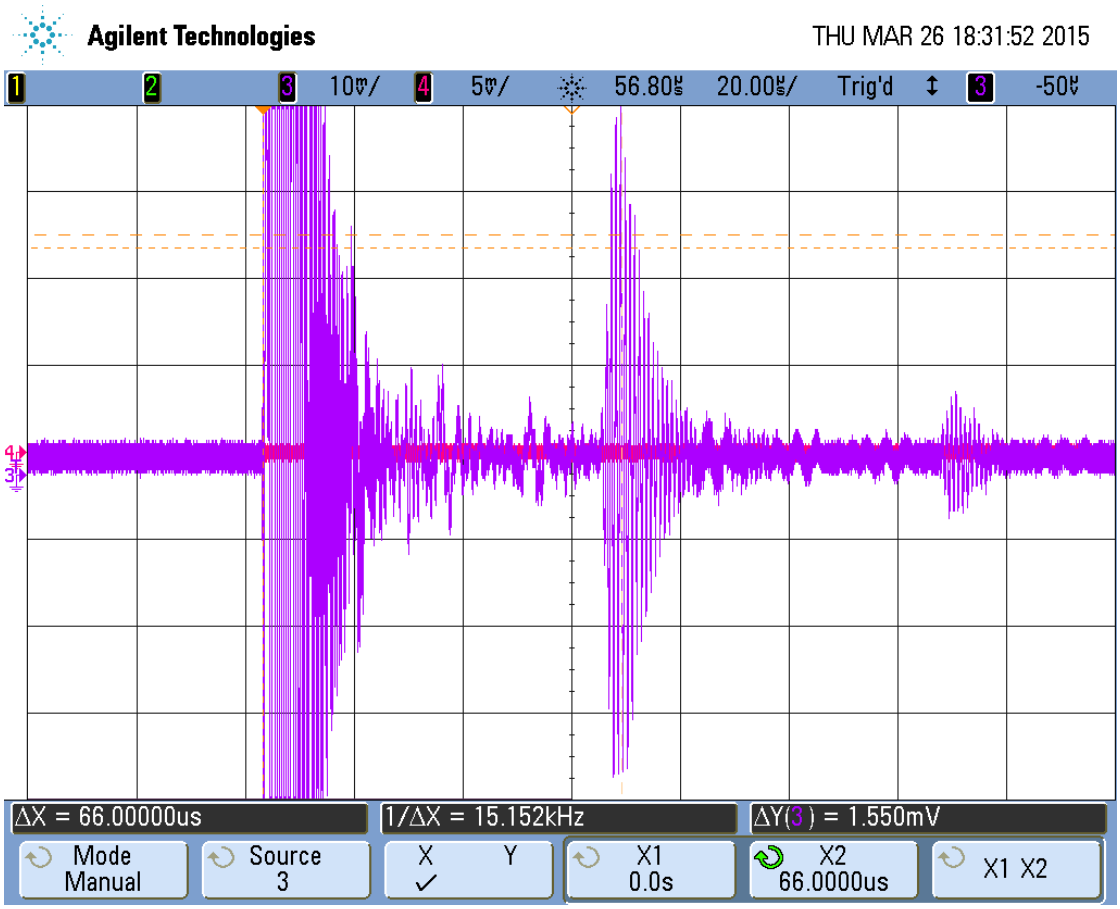

Figure A-3: HIFU focal point using pulsar receiver

A-4 Clot tables for 12 individual clots

Initial clot weight : 43mg										
Driving voltage of HIFU (Function generator)	PNP(MPa)	Movement				Cavitation events				
		No	Slow	Swirling	Violent	Count 1	Count 2	Count 3	Avg	Count classification
100	0.28								0	L
200	0.68								0	L
300	1.18								0	L
400	1.53	✓				0	0	0	0	L
500	2.08	✓				33	0	1	11	L
600	2.42		✓			4924	8807	4112	5948	L
700	2.71				✓	67332	56000	61681	61671	H
800	3.03				✓	87379			87379	H
900	3.18									
Final clot weight : 0										
Thrombolysis : 100%										

Figure A-4: Clot 1

Initial clot weight: 30mg

Driving voltage of HIFU (Function generator)	PNP(MPa)	Movement				Cavitation events				
		No	Slow	Swirling	Violent	Count 1	Count 2	Count 3	Avg	Count classification
100	0.28								0	L
200	0.68								0	L
300	1.18								0	L
400	1.53	✓				1	0	0	0	L
500	2.08	✓				17	10	31	19	L
600	2.42		✓			9678	8919	8525	9041	L
700	2.71			✓		68781	81114	80661	76852	H
800	3.03				✓	83187	87679	90202	87023	H
900	3.18									

Final clot weight : 0
Thrombolysis 100%

Figure A-5: Clot 2

Initial clot weight : 28mg

Driving voltage of HIFU (Function generator)	PNP(MPa)	Movement				Cavitation events				
		No	Slow	Swirling	Violent	Count 1	Count 2	Count 3	Avg	Count classification
100	0.28								0	L
200	0.68								0	L
300	1.18								0	L
400	1.53								0	L
500	2.08		✓			882	812	910	868	L
600	2.42		✓			69850	8359	56082	44764	M
700	2.71			✓		81261	86723	87944	85309	H
800	3.03				✓	90337	78152	92053	86847	H
900	3.18									

Final clot weight : 0
Thrombolysis : 100%

Figure A-6: Clot 3

Initial weight : 22mg

Driving voltage of HIFU (Function generator)	PNP(MPa)	Movement				Cavitation events				
		No	Slow	Swirling	Violent	Count 1	Count 2	Count 3	Avg	Count classfication
100	0.28								0	L
200	0.68								0	L
300	1.18								0	L
400	1.53								0	L
500	2.08	✓				1	1	1	1	L
600	2.42		✓			39995	41214	23284	34831	M
700	2.71			✓		88121	89174	91288	89528	H
800	3.03			✓		90591	92036	91159	91262	H
900	3.18			✓		95765	92970	92965	93900	H

Final weight : 3mg
Thrombolysis :86%

Figure A-7: Clot 4

Initial weight : 79mg

Driving voltage of HIFU (Function generator)	PNP(MPa)	Movement				Cavitation events				
		No	Slow	Swirling	Violent	Count 1	Count 2	Count 3	Avg	Count classification
100	0.28								0	L
200	0.68								0	L
300	1.18								0	L
400	1.53								0	L
500	2.08	✓				6	1	0	2	L
600	2.42		✓			67067	65043	48245	60118	H
700	2.71		✓			70531	72250	71734	71505	H
800	3.03				✓	74306	73465	66128	71300	H
900	3.18				✓	61624	66074	63665	63788	H

Final weight : 0
Thrombolysis : 100%

Figure A-8: Clot 5

Initial weight : 29mg

Driving voltage of HIFU (Function generator)	PNP(MPa)	Movement				Cavitation events				
		No	Slow	Swirling	Violent	Count 1	Count 2	Count 3	Avg	Count classification
100	0.28								0	L
200	0.68								0	L
300	1.18								0	L
400	1.53								0	L
500	2.08	✓				3	3	25	10	L
600	2.42			✓		0	0	319	106	L
700	2.71				✓	61849	64727	57188	61255	H
800	3.03									
900	3.18									

Final weight : 2mg
Thrombolysis : 93%

Figure A-9: Clot 6

Initial clot weight : 61mg

Driving voltage of HIFU (Function generator)	PNP(MPa)	Movement				Cavitation events				
		No	Slow	Swirling	Violent	Count 1	Count 2	Count 3	Avg	Count classification
100	0.28								0	L
200	0.68								0	L
300	1.18								0	L
400	1.53								0	L
500	2.08	✓				124	2	0	42	L
600	2.42			✓		40292	43674	22150	35372	M
700	2.71				✓	67773	64839	71467	68026	H
800	3.03								0	
900	3.18								0	

Final clot weight :0
Thrombolysis: 100%

Figure A-10: Clot 7

Initial weight : 77mg

Driving voltage of HIFU (Function generator)	PNP(MPa)	Movement				Cavitation events				Count classification
		No	Slow		Violent	Count 1	Count 2	Count 3	Avg	
100	0.28								0	L
200	0.68								0	L
300	1.18								0	L
400	1.53								0	L
500	2.08	✓				8	0	0	3	L
600	2.42		✓			3521	11028	17306	10618	L
700	2.71		✓			62231	66535	67082	65283	H
800	3.03		✓			76297	78701	75865	76954	H
900	3.18				✓	74752	87164	74704	78873	H

Final weight : 8mg
Thrombolysis: 89%

Figure A-11: Clot 8

Initial weight : 26mg

Driving voltage of HIFU (Function generator)	PNP(MPa)	Movement				Cavitation events				Count classification
		No	Slow	Swirling	Violent	Count 1	Count 2	Count 3	Avg	
100	0.28								0	L
200	0.68								0	L
300	1.18								0	L
400	1.53								0	L
500	2.08	✓				2	0	0	1	L
600	2.42		✓			7046	5048	2829	4974	L
700	2.71			✓		43096	43195	74930	53740	M
800	3.03				✓	89653			29884	H
900	3.18									

Final weight : 0
Thrombolysis : 100%

Figure A-12: Clot 9

Initial clot weight 88mg

Driving voltage of HIFU (Function generator)	PNP(MPa)	Movement				Cavitation events				Count classification
		No	Slow	Swirling	Violent	Count 1	Count 2	Count 3	Avg	
100	0.28								0	L
200	0.68								0	L
300	1.18								0	L
400	1.53								0	L
500	2.08	✓				74	261	238	191	L
600	2.42		✓			46	22	25	31	L
700	2.71			✓		5368	13587	75789	31581	M
800	3.03				✓	88100			88100	H
900	3.18									

Final clot weight : 0
Thrombolysis : 100%

Figure A-13: Clot 10

Initial clot weight: 66mg

Driving voltage of HIFU (Function generator)	PNP(MPa)	Movement				Cavitation events				Count classification
		No	Slow	Swirling	Violent	Count 1	Count 2	Count 3	Avg	
100	0.28								0	L
200	0.68								0	L
300	1.18								0	L
400	1.53								0	L
500	2.08	✓				1	2	2	2	L
600	2.42			✓		2481	449	435	1122	L
700	2.71			✓		6504	1501	612	2872	L
800	3.03				✓	36893	62541		33145	H
900	3.18									

Final clot weight : 2mg
Thrombolysis: 97%

Figure A-14: Clot 11

Initial weight : 50mg

Driving voltage of HIFU (Function generator)	PNP(MPa)	Movement				Cavitation events				Count classfication
		No	Slow	Swirling	Violent	Count 1	Count 2	Count 3	Avg	
100	0.28								0	L
200	0.68								0	L
300	1.18								0	L
400	1.53								0	L
500	2.08	✓				2	0	11	4	L
600	2.42		✓			20	16	19	18	L
700	2.71			✓		421	858	730	670	L
800	3.03			✓		4583	12564	12691	9946	L
900	3.18			✓		63724	84268	80568	76187	H

Final weight : 34 mg

Figure A-15: Clot 12

Bibliography

[1] "Heartmate ii lvas, left ventricular assist system operating manual," 2005.

[2] M. S. Slaughter, J. G. Rogers, C. A. Milano, S. D. Russell, J. V. Conte, D. Feldman, B. Sun, A. J. Tatooles, R. M. Delgado, J. W. Long, T. C. Wozniak, W. Ghumman, D. J. Farrar, and O. H. Frazier, "Advanced heart failure treated with continuous-flow left ventricular assist device," *New England Journal of Medicine*, vol. 361, no. 23, pp. 2241–2251, 2009. PMID: 19920051.

[3] N. Uriel, K. A. Morrison, A. R. Garan, T. S. Kato, M. Yuzefpolskaya, F. Latif, S. W. Restaino, D. M. Mancini, M. Flannery, H. Takayama, R. John, P. C. Colombo, Y. Naka, and U. P. Jorde, "Development of a Novel Echocardiography Ramp Test for Speed Optimization and Diagnosis of Device Thrombosis in Continuous-Flow Left Ventricular Assist DevicesThe Columbia Ramp Study," *Journal of the American College of Cardiology*, vol. 60, pp. 1764–1775, Oct. 2012.

[4] S. S. Najjar, M. S. Slaughter, F. D. Pagani, R. C. Starling, E. C. McGee, P. Eckman, A. J. Tatooles, N. Moazami, R. L. Kormos, and D. R. Hathaway, "An analysis of pump thrombus events in patients in the HeartWare ADVANCE bridge to transplant and continued access protocol trial," *The Journal of Heart and Lung Transplantation*, vol. 33, no. 1, pp. 23–34, 2014.

[5] N. M. Tole, *Basic Physics of ultrasonographic imaging* . 2005.

[6] I. Lentacker, S. C. De Smedt, and N. N. Sanders, "Drug loaded microbubble design for ultrasound triggered delivery," *Soft Matter*, vol. 5, pp. 2161–2170, 2009.

[7] S. Baldasseroni, C. Opasich, M. Gorini, D. Lucci, N. Marchionni, M. Marini, C. Campana, G. Perini, A. Deorsola, and G. Masotti, "Left bundle-branch block is associated

with increased 1-year sudden and total mortality rate in 5517 outpatients with congestive heart failure: a report from the Italian network on congestive heart failure," *American heart journal*, vol. 143, no. 3, pp. 398–405, 2002.

[8] E. Friedrich and M. Böhm, "Management of end stage heart failure," *Heart*, vol. 93, no. 5, pp. 626–631, 2007.

[9] L. E. Rodriguez, E. E. Suarez, M. Loebe, and B. A. Bruckner, "Ventricular Assist Devices (VAD) Therapy: New Technology, New Hope?," *Methodist DeBakey cardiovascular journal*, vol. 9, no. 1, p. 32, 2013.

[10] D. J. Goldstein, M. C. Oz, and E. A. Rose, "Implantable left ventricular assist devices," *New England Journal of Medicine*, vol. 339, no. 21, pp. 1522–1533, 1998.

[11] D. R. Kumar, E. Hanlin, I. Glurich, J. J. Mazza, and S. H. Yale, "VirchowâĂŹs Contribution to the Understanding of Thrombosis and Cellular Biology," *Clin Med Res*, vol. 8, no. 3-4, pp. 168–172, 2010.

[12] M. R. Mehra, G. C. Stewart, and P. A. Uber, "The vexing problem of thrombosis in long-term mechanical circulatory support," *The Journal of Heart and Lung Transplantation*, vol. 33, no. 1, pp. 1–11, 2014.

[13] R. C. Starling, Y. Naka, A. J. Boyle, G. Gonzalez-Stawinski, R. John, U. Jorde, S. D. Russell, J. V. Conte, K. D. Aaronson, E. C. McGee, *et al.*, "Results of the post-us food and drug administration-approval study with a continuous flow left ventricular assist device as a bridge to heart transplantation: a prospective study using the intermacs (interagency registry for mechanically assisted circulatory support)," *Journal of the American College of Cardiology*, vol. 57, no. 19, pp. 1890–1898, 2011.

[14] T. R. Porter and F. Xie, "Ultrasound, microbubbles, and thrombolysis," *Progress in cardiovascular diseases*, vol. 44, no. 2, pp. 101–110, 2001.

[15] B. Petit, E. Gaud, D. Colevret, M. Arditi, F. Yan, F. Tranquart, and E. Allémann, "Sonothrombolysis of Human Blood Clots with BR38 Microbubbles," *Ultrasound in medicine & biology*, vol. 38, no. 7, pp. 1222–1233, 2012.

[16] T. R. Porter, "The utilization of ultrasound and microbubbles for therapy in acute coronary syndromes," *Cardiovascular research*, vol. 83, no. 4, pp. 636–642, 2009.

[17] S. Datta, C.-C. Coussios, A. Y. Ammi, T. D. Mast, G. M. de Courten-Myers, and C. K. Holland, "Ultrasound-enhanced thrombolysis using definity® as a cavitation nucleation agent," *Ultrasound in medicine & biology*, vol. 34, no. 9, pp. 1421–1433, 2008.

[18] J. E. Leeman, J. S. Kim, F. T. H. Yu, X. Chen, K. Kim, J. Wang, X. Chen, F. S. Villanueva, and J. J. Pacella, "Effect of acoustic conditions on microbubble-mediated microvascular sonothrombolysis," *Ultrasound in medicine & biology*, vol. 38, no. 9, pp. 1589–1598, 2012.

[19] T. Li, H. Chen, T. Khokhlova, Y. N. Wang, W. Kreider, X. He, and J. H. Hwang, "Passive cavitation detection during pulsed HIFU exposures of ex vivo tissues and in vivo mouse pancreatic tumors," *Ultrasound Med Biol*, vol. 40, no. 7, pp. 1523–1534, 2014.

[20] K. B. Bader, M. J. Gruber, and C. K. Holland, "Shaken and Stirred: Mechanisms of Ultrasound-Enhanced Thrombolysis," *Ultrasound in Medicine and Biology*, vol. 41, pp. 187–196, July 2015.

[21] M. J. Kern, J. A. Moore, F. V. Aguirre, R. G. Bach, E. A. Caracciolo, T. Wolford, A. F. Khoury, C. Mechem, and T. J. Donohue, "Determination of angiographic (TIMI grade) blood flow by intracoronary Doppler flow velocity during acute myocardial infarction.," *Circulation*, vol. 94, no. 7, pp. 1545–1552, 1996.

[22] W. Steffen, M. C. Fishbein, H. Luo, D.-Y. Lee, H. Nitams, D. C. Cumberland, S. W. Tabak, M. Carbonne, G. Maurer, and R. J. Siegel, "High intensity, low frequency catheter-delivered ultrasound dissolution of occlusive coronary artery thrombi: an in vitro and in vivo study," *Journal of the American College of Cardiology*, vol. 24, no. 6, pp. 1571–1579, 1994.

[23] M. Ariani, M. C. Fishbein, J. S. Chae, H. Sadeghi, A. D. Michael, S. B. Dubin, and R. J. Siegel, "Dissolution of peripheral arterial thrombi by ultrasound," *Circulation*, vol. 84, no. 4, pp. 1680–1688, 1991.

[24] U. Rosenschein, V. Furman, E. Kerner, I. Fabian, J. Bernheim, and Y. Eshel, "Ultrasound ImagingâĂŞGuided Noninvasive Ultrasound Thrombolysis Preclinical Results," *Circulation*, vol. 102, no. 2, pp. 238–245, 2000.

[25] K. Tachibana and S. Tachibana, "Albumin microbubble echo-contrast material as an enhancer for ultrasound accelerated thrombolysis.," *Circulation*, vol. 92, no. 5, pp. 1148–1150, 1995.

[26] C. Damianou, V. Hadjisavvas, N. Mylonas, A. Couppis, and K. Ioannides, "MRI-Guided Sonothrombolysis of Rabbit Carotid Artery," *Journal of Stroke and Cerebrovascular Diseases*, 2013.

[27] J. Eggers, S. Ossadnik, and G. Seidel, "Enhanced Clot Dissolution by 1.8-MHz Pulsed Ultrasound," *Ultrasound in medicine & biology*, vol. 35, no. 3, pp. 523–526, 2009.

[28] T. Nishioka, H. Luo, M. C. Fishbein, B. Cercek, J. S. Forrester, C.-J. Kim, H. Berglund, and R. J. Siegel, "Dissolution of thrombotic arterial occlusion by high intensity, low frequency ultrasound and dodecafluoropentane emulsion: an in vitro and in vivo study," *Journal of the American College of Cardiology*, vol. 30, no. 2, pp. 561–568, 1997.

[29] Y. Birnbaum, H. Luo, T. Nagai, M. C. Fishbein, T. M. Peterson, S. Li, D. Kricsfeld, T. R. Porter, and R. J. Siegel, "Noninvasive in vivo clot dissolution without a thrombolytic drug recanalization of thrombosed iliofemoral arteries by transcutaneous ultrasound combined with intravenous infusion of microbubbles," *Circulation*, vol. 97, no. 2, pp. 130–134, 1998.

[30] S. Xu, Y. Zong, Y. Feng, R. Liu, X. Liu, Y. Hu, S. Han, and M. Wan, "Dependence of pulsed focused ultrasound induced thrombolysis on duty cycle and cavitation bubble size distribution," *Ultrasonics sonochemistry*, vol. 22, pp. 160–166, 2015.

[31] E. L. Carstensen, W. K. Law, N. D. McKay, and T. G. Muir, "Demonstration of nonlinear acoustical effects at biomedical frequencies and intensities.," *Ultrasound in medicine & biology*, vol. 6, no. 4, pp. 359–368, 1980.

[32] X. Chen, J. Leeman, J. Wang, J. Pacella, and F. Villanueva, "New Insights into Mechanisms of Sonothrombolysis Using Ultra-High-Speed Imaging," *Ultrasound in medicine & biology*, vol. 40, no. 1, pp. 258–262, 2014.

[33] J. T. Sutton, N. M. Ivancevich, S. R. Perrin, D. C. Vela, and C. K. Holland, "Clot Retraction Affects the Extent of Ultrasound-Enhanced Thrombolysis in an Ex Vivo Porcine Thrombosis Model," *Ultrasound in Medicine and Biology*, vol. 39, no. 5, pp. 813–824, 2013.