Effect of Vibration imposition on Aortic Muscle Tissue : An Explorative Study

A THESIS SUBMITTED TO AUCKLAND UNIVERSITY OF TECHNOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

Under the supervision of

Dr. Jun Lu

Dr. Andrew Lowe

2017

By

Subodh Ajgaonkar

Auckland University of Technology

School of Interprofessional Health Studies and

School of Engineering.

Copyright

Declaration

I hereby declare that this submission is my own work and that to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the qualification of any other degree or diploma of a university or other institution of higher learning

Subodh Ajgaonkar

Acknowledgements

There are so many people I would like to thank for helping me academically, financially and emotionally in the past one year, without whom this work would not have been possible. Many people have made my stay in Auckland easier for me and have supported me thru this journey of my Masters.

First and foremost, I would like to thank **Dr. Jun Lu** for his guidance, encouragement and patience over the last year. Thank you, Dr. Lu, for giving me an opportunity to work with such a project and forcing me to look at and develop new ways to complete my studies. Your support was essential for my success.

I would also like to thank **Dr. Andrew Lowe**, for talking to me on various occasions and for his support and encouragement. Thank you, Dr. Lowe, for working out my results as well and helping me with all the calculations.

My sincere gratitude and a heartfelt thanks to **Dr. Miguel Jo-Avila**, for being my support and for working out with me the initial protocol, obtaining the animals and the tissue sample and the statistical analysis of my research. Thank you, Miguel, for your patience, help and composure which made the entire research possible.

Tanvi Nagari, you have been a pillar of support and patience since I started with my Masters. Your help in the most important part of my experimentation and results was necessary and needed to complete my degree. If it had not been you, I would still be trying to just make sense of my results. Your support, encouragement and friendship are what have kept me going through my Masters journey. Thank you so much for your sacrifices, and being their whenever I needed you to be there.

I would like to sincerely thank from the bottom of my heart my family, especially my mother **Mrs. Neeta Ajgaonkar**, who taught me to face and overcome challenges no matter how difficult, and my father **Mr. Datta Ajgaonkar** for never letting me give up on my dreams no matter how bleak the situation got. Thank you for motivating me and guiding me to become more knowledgeable and educated and making me wiser for my future and the impending decisions.

Finally, I would like to thank **Mr. Kevin Roos**, for supporting me in absence of Miguel and for accommodating my needs for animals and for the issues I caused in the lab. I will also take this chance to thank all my friends in New Zealand who have been there for me and have kept me sane all this time.

Abstract:

Aorta, the main artery of the human body has a pipe like structure and is tasked with carrying and distributing the oxygenated blood to the various parts of the human body. Development of small kink or obstruction, clinically also called as the Coarctation of Aorta (CoA) is a congenital condition leading to narrowing of the aorta. This generally tends to happen to the left side of the heart. Coarctation or narrowing of the aorta leads to increase in the active forces along the walls of aorta and high blood pressure. This study tends to research the effect of vibration imposition on aorta muscle tissue to decrease the active forces along its walls and eventually lead to its relaxation.

Sinusoidal vibrations are a type of smooth vibrations have shown to decrease the active forces seen muscle tissues and subsequently relax the tissue. Relaxation of other smooth tissue samples as that from Airway smooth muscle and trachea have given credence to the hypothesis. This hypothesis however has not yet been applied to the aortic smooth muscle.

Thus, this research focuses on relaxation of smooth muscles – particularly those of rat aorta using smooth vibrations. The aim of the research is to find a frequency of vibrations that can relax maximally constricted rat aortic tissue and reduce active muscle forces. It can then be expanded to include various smooth muscle tissues leading to better management of cardiovascular diseases. In this study, increasing relaxation in tissues was seen with a subsequent increase in the frequency of the vibrations. The maximum relaxation seen was at 24% of initial stress for 50hz frequency. The study also shows a gradual increase in relaxation across various frequencies, starting from 10% for 20hz to 24% for 50hz. It can also be inferred from the study's result that there is a decrease in relaxation from the first vibration imposition cycle to next and thus, there is a probability of tissue fatigue. The relaxations indicate the effect sinusoidal vibrations have on the stress level and the active forces of the aorta tissue sample.

Table of Contents

Table of Images and Tables

Chapter 1

Introduction

This section introduces the basic idea behind the research and the diseases and conditions associated with it. It explains in depth the cardiovascular system, aorta and the Coarctation of aorta, one of the most common disease that leads to an increase in active forces of the aorta and its contraction.

1.1 – Cardiovascular System and Background:

The cardiovascular system, or in other words the circulatory system is an organ system that permits the flow of nutrients such as amino acids and electrolytes along with oxygen, carbon dioxide, hormones and blood to and from the cells. This provides nourishment, ability to fight diseases and heal from wounds, stabilize temperature with pH and maintain homeostasis. This makes it one of the most important organ system in the human body. The cardiovascular system comprises of blood, heart and blood vessels. One of the most important components of the cardiovascular system is the aorta which is the primary artery. It distributes oxygenated blood through the human body and is essential for maintaining a normal blood pressure (Guyton & Hall, 2000)

The cardiovascular system consists of the heart, lungs, arteries -blood vessels providing oxygenated blood and capillaries – branches of the arteries supplying blood to the venous system and the veins – blood vessels bringing deoxygenated blood back to the heart. Arteries arise out of the left ventricle of the heart leading to formation of blood vessels down the body which then congregate to form the veins, thus completing a cycle (Martin, 2015; Stöllberger, 2014). The cardiovascular system consists of different “loops” that circulate the blood throughout the body and cycle between oxygenated and deoxygenated blood. Different “loops” of the circulatory system would be the circulation of blood where the blood flows through lungs and is oxygenated, and the systemic circulation “loop” which provides the entire body with this blood.

However, many diseases affect the cardiovascular system which are commonly known as cardiovascular diseases. These diseases are then categorized as either congenital diseases i.e diseases affecting patients from birth and "lifestyle diseases" that are caused over time by lack of physical activities, sedentary lifestyle and person's habits. However, most of these diseases share common symptoms such as high blood pressure, higher risk of stroke and higher chances of aneurysm. The current techniques to treat such diseases are based on drugs either through the oral or intravenous route or through surgery.

1.2- Aorta:

The aorta is a massive thick-walled artery that originates from the left ventricle of the heart and branches further down the body. Initially, the aorta arches and branches to supply blood to the upper body, before entering the abdomen, where it further branches to provide blood to the abdomen, pelvis and the lower limbs (Cui, 2011; Fiore & Schmidt, 1974). According to Fiore and Schmidt (1974), the walls of aorta are elastic which is the main reason for its ability to maintain the blood pressure throughout the body. The aorta after receiving blood from heart recoils leading to pulsating blood pressure and then transports this blood throughout the body with the help of capillaries. As the aorta branches into capillaries its elasticity decreases.

Anatomically the aorta is divided into sections such as the ascending aorta – the region of aorta from the left ventricle of the heart to thoracic region, the thoracic aorta – from the thorax to the diaphragm and the abdominal aorta – the region of the aorta in the abdominal or the mid-section of the body(Drake, Vogl, Mitchell, Gray, & Based on : Gray, 2010; Grotenhuis & de Roos, 2011; Netter, 2014) . Histologically, it's a mixture of smooth muscle cells, nerves, endothelial cells, fibroblast like cells and an extracellular matrix. It is also lined by the baroreceptors and chemoreceptors that relay the blood pH, blood pressure and carbon dioxide levels back to the brain.

The aorta is not a simple tube that carries oxygenated blood, but highly specialized and complex part of cardiovascular diseases. It maintains and performs function related to the left ventricle (Lang, 1997), myocardial perfusion and arterial functions for the entire cardiovascular system (Nichols, O'Rourke, & Vlachopoulos, 2011). The various branches of the aorta – the thoracic and the abdominal – perform different functions of supplying blood to in relation to their placement. Its elastic nature introduces pulses through the aorta that propagates throughout the

aorta leading to blood flow and maintaining the blood pressure. Hence, it can be inferred that the elastic nature of aorta and its walls is essential to main a normal blood pressure and play an important role in maintaining normal flow of blood in the body.

1.3 – Coarctation of Aorta and Active forces:

As mentioned earlier, CoA or in simpler terms narrowing of aorta is a congenital condition. It usually occurs very commonly in the aortic arch. Coarctation of aorta also involves the stiffening of the aortic walls followed by abnormal distribution of blood in the lower half of the body. The left ventricle of the heart pumps blood throughout the body and hence maintains blood pressure. In people with Coarctation of aorta, the left ventricle must generate more pressure to maintain normal blood pressure. This however, adversely effects the walls of the aorta leading to increase in stress.

In people with Coarctation of aorta, the left ventricle works harder to maintain blood pressure and the flow of blood around the body. Also, since the aorta is narrow, the left ventricle must generate a lot more force to pump the blood through the body. If the aorta in CoA is narrow enough, it might lead to the lack of blood in the lower regions of the body. Currently CoA is treated with either with surgery or with drugs. The surgical process is common and involves either placement of stents or angioplasty to dilate the narrowed aorta. However, all the treatments methods for CoA are not localized and affect the aorta instead of just the narrow area. Using vibrations, the treatment can be much more localized and focused on the area of interest. It would also not affect the entire aorta, thus weakening it. Thus, one of the other aims of my research is also to find a more localized treatment for CoA and better management of cardiovascular diseases in general.

In this study, smooth vibration or sinusoidal vibrations were applied on the aortic rat muscles that were constricted maximally using chemical stimuli (Potassium Chloride Solution) and the effect of the vibration was studied. In the first stage of the research, different chemical stimuli to constrict the tissue sample were evaluated based on literature research and their effectiveness in constricting the aorta. Vibrations were applied on the constricted aortic rings to relax them and the difference between the contractions and relaxations were observed and difference between them were noted. This research has thus proved that when aortic tissue sample

are applied with smooth vibration, a continuous reduction in their active forces is seen leading to their relaxation.

1.4 – Sinusoidal vibrations:

Sinusoidal vibrations are the simplest form of vibration which are periodic and smooth. In sinusoidal vibration, the body which would be exposed to vibration, moves around the equilibrium in a smooth, periodic and controlled way. An ideal and perfect example of sinusoidal vibration would be a load or a mass attached to a spring and subject to no friction. The reasons as to why sinusoidal vibrations were chosen for this study are,

1. Sinusoidal vibrations as mentioned earlier are periodic and smooth. Hence, it is easy to convert overall values between peak, peak to peak and rms. It is also easy to convert between acceleration, velocity and displacement.
2. Any waveform no matter how complex can be decomposed to sinusoidal vibrations and is the base of frequency analysis.
3. Sinusoidal vibrations are smooth and hence there is no unnecessary stress on the tissue sample.

The general equation for a sinusoidal waveform would be,

$$x(t) = X \cdot \cos(2\pi ft - \varphi)$$

where, X is the amplitude, f is the frequency and φ is the phase. For this study only the frequency changes while the other parameters are kept constant. A graphical representation of sinusoidal wave is as follows,

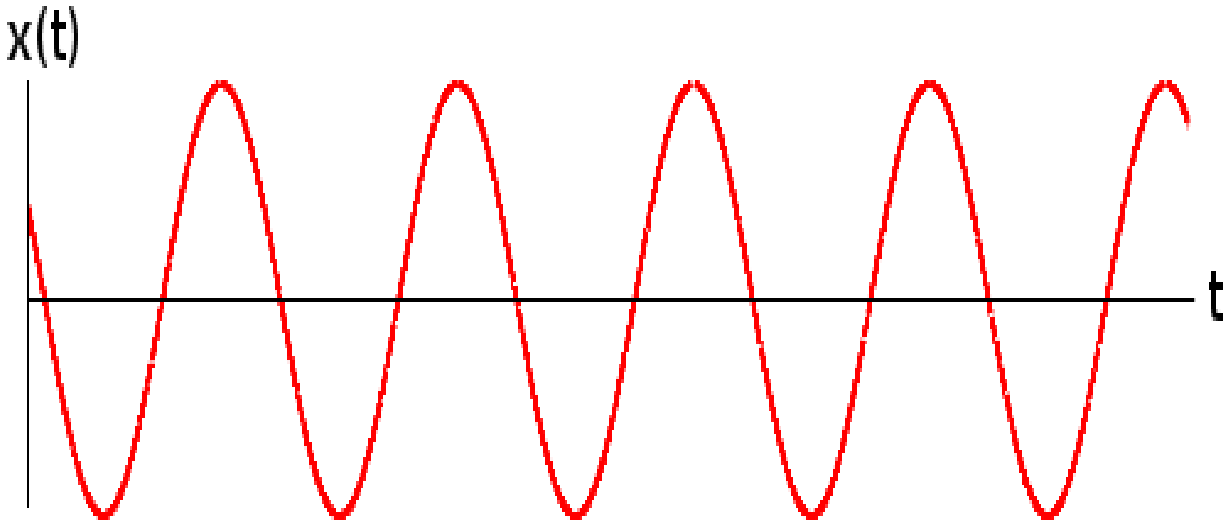

Figure 1 : A Classic sinusoidal waveform.

1.5 – Aims and Objectives:

The main aim of this study is to research and find the effect of vibration imposition on aortic tissue sample. Some evidence exists to show relaxing effect of vibrations on other sample of the tissues such as trachea and Airway Smooth Muscle (ASMs). This study aims to replicate the results and effects of those studies on aorta tissue which is yet to be studied. Various frequencies with a gap of 10hz are selected to impose on tissue sample.

The other objective of this study was to research the physical effects of vibrations on the tissue sample. Preliminary tests conducted on the tissue sample indicate decrease in the relaxation following one cycle to other. It remains to be seen if this is the case with all the frequencies that would be imposed on the sample. To summarize, the aim and objectives of this study is,

- Study the relaxation effect of imposing vibration on aortic tissue sample.
- Study the physical effect of vibration on tissue samples
- Study the morphological changes tissue undergoes because of the vibration imposition if possible.

Chapter 2

Literature Review

This section introduces the previous research done for relaxation of smooth muscle tissue using frequencies. While there are some similarities in the other work about relaxation, certain differences are seen as well which have been explained and analyzed below. Since literature for aortic contraction and relaxation is rare, this research takes a drawing from works around ASMs. Preliminary test for this study have shown that muscle tissue samples tend to relax when exposed to vibrations. Additional research by Ijpma, Al-Jumaily, Cairns, and Sieck (2010) and Du, Al-Jumaily, and Shukla (2007) has shown that smooth muscle tissues like airway smooth muscle and trachea relax when imposed with vibration of particular frequency.

2.1 – Chemical Stimuli and Vibration frequency:

When previous works were compared, some shortcomings were evident. The work of Ijpma et al. (2010) investigated the imposition of frequency on relaxed porcine airways smooth muscle (ASM) to draw the conclusion that, smooth muscle show a decrease in active forces once imposed with frequency of a particular frequency. The notable differences between the said research and my study would be inclusion of muscle tissue with contractions chemically stimulated in it. Furthermore, the chemical used to contract the ASMs was Acetylcholine (AcH) while the work of Angus, Cocks, McPherson, and Broughton (1991); Furchgott and Zawadzki (1980) have shown that AcH acts as a vasodilator or in simpler terms a relaxant when used with Endothelium intact (E+) samples. Since, for this study, the aorta sample would be untouched, Potassium Chloride (KCl) would be used as contracting agent (Pérez-Vizcaíno, Fernández del Pozo, Zaragozá, & Tamargo, 1994).

Statistically, contracted ASMs have shown decrease in stiffness when imposed with vibrations within the range of 10-100 Hz maximizing out at around 25 Hz (Du et al., 2007). This has proved that when externally contracted ASMs are subjected to longitudinal vibrations with various frequencies, amplitude and duration changes are seen in them. The works by Du et al.

(2007); Ijpma et al. (2010) have also shown, as mentioned beforehand, 25 -30 Hz maximize relaxation and hence for the aorta tissue this would be the frequency to focus on.

With ASMs, another important factor to consider would be tidal breathing. Tidal breathing generates vibrations which can have either an adverse effect or a positive effect on the relaxation of ASMs by vibration imposition. Studies such as those by Burns and Gibson (1998); Gunst and Wu (2001); Wang and Pare (2003) have shown that ASMs relax when imposed with frequencies similar to those of tidal breathing. This hints at factoring of blood pressure and heart beat might be needed to study the full extent of relaxation by frequency imposition.

2.2 – Smooth muscle structure and biomechanical properties:

Previous studied have all researched on smooth muscle cells and imposition of vibration on those smooth muscle cells. Hence, understanding the structure of smooth muscle cells and aorta becomes important. Another important reason to study the structure of smooth muscle would be because they govern the biomechanical and physical properties of the tissue.

Smooth muscle cells have a plasma membrane around 80 A thick which surrounds the entire cell (Matthews & Gardner, 1966; Ohlstein & Douglas, 1993; Rhodin, 1962; Somlyo & Somlyo, 1968). Evidence of cytoplasmic continuity between different cells is not seen in such smooth muscle cells and vascular smooth muscle cells do not form true anatomic syncytium. The connective tissue component separating smooth muscle cells of different vessels and vascular collagen content tends to increase with age (Cliff, 1967)

Another important and distinctive feature of smooth muscle is the presence of vesicles. Vesicles are spherical invagination of plasma membrane ranging between 450A(Matthews & Gardner, 1966) and 800A (Cliff, 1967). Vesicles are important because they transport vital nutrients such as Calcium and Phosphorus to the cell and play an important role in their contraction (Reynolds et al., 2004). Also, the number and distribution of the vesicles represent the functional state of the tissue. Vesicles are found in greater number in the endothelial smooth muscle cells (Dowe, Fioranelli, & Pavone, 2013; McManaman, Reyland, & Thrower, 2006) to the outer adventive surface (Lever, Ahmed, & Irvine, 1965) when the tissue is in relaxed state.

The biomechanical properties of the muscle are due to smooth muscle cells (Fabry & Fredberg, 2003; Xavier Trepat et al., 2007; X. Trepat et al., 2004) and hence control the contractions and relaxations of the muscle. Along with the smooth muscle cells, epithelial cells play an important role in stretching and relaxing the muscle sample. Hence consideration and study of both becomes important during consideration of muscle contraction.

The biomechanical properties conferred to a muscle because of smooth cells are due to two contractile components present in them – the myofilament and the dense bodies. The contraction and relaxation of a smooth muscle is because of the movement and interconnection of these two. The contractile forces originate from cross bridge of H-meromyosin head of the myosin molecule which makes up the thick filament (Poole et al., 2006; Zoghbi, Woodhead, Moss, & Craig, 2008) and the adjacent thin filament containing actin and native tropomyosin (Gerthoffer, 2005). The image below shows contraction and relaxation in a muscle and the movement of the filaments in that regards.

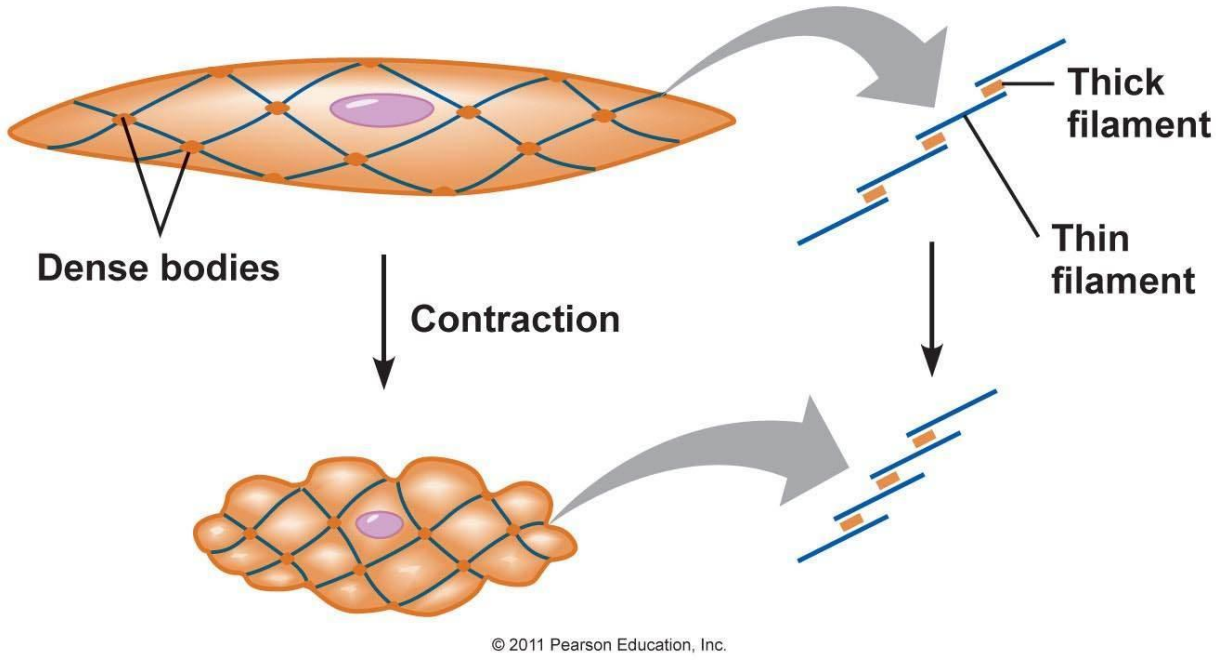

*Figure 2 : Muscle Contraction and Relaxation (source - http://www.keywordsuggests.com/pyUrOBOy8yA*W*spCDwpH4sFe5I275tc8NTmEpjvfrs/).*

Myofilaments are the characteristic cytoplasmic component of the smooth muscles which are densely packed in the peripheral area of the cytoplasm and run irregularly but on average parallel to the axis of muscle fiber. Myofilaments can be observed clearly and better in a relaxed muscle than in contracted muscle, because they play a role in contracting the muscle mass by applying force and tend to decrease in size. Myofilaments are often seen as that of different sizes and shape depending on the muscle and the location as that in bovine carotid artery, where two distinct sets are seen, one with a diameter of 90A and the other with 120A.

2.3 – Muscle Contraction Mechanism:

Dense bodies on the other hand are linked intermittently and intimately to the myofilament. Dense bodies stain dark and are spindle shaped areas which vary in length from 4000- 9000A and in width from 2000-5000A (Matthews & Gardner, 1966). Myofilaments shorten and enter dense bodies during the muscle contraction and the plasma membrane of the dense bodies seem retracted (Somlyo & Somlyo, 1968). Another important function of these dense bodies is formation of area for increased contractile material from the overlap of thin actin filaments and thick myosin filaments.

During muscle contractions, tension generating sites are activated on the muscle (Silverthorn, Johnson, Ober, Garrison, & Silverthorn, 2013; Widmaier, Raff, & Strang, 2008). When muscles contract, they do not shorten in size nor change their structure. This is because muscle tension can also be generated without changing the muscle size such as when holding a heavy object. Muscle contractions are then followed by muscle relaxation which is a return to their original low tension generating state. Muscle contraction is important from the point of view that it can be described using two variables: tension and length (Widmaier et al., 2008) which are used in place of one another for this experiment.

Muscle contractions are of different types such as isometric – when muscle tension changes and length stays constant or isotonic when length changes but tension stays constant (Aidley, 1978; Rhoades & Bell, 2009). For cardiac muscle to consider their contractions, other factors such as temperature and Oxygen intake also needs to be considered (Gibbs & Chapman, 1985; Rall & Woledge, 1990). However, this experiment deals with aortic muscle tissue now and hence, to consider these parameters would increase the scope of the experiment.

Chapter 3

Preliminary Investigation and Experimental Protocol

This section details out the steps taken to confirm if the vibrations had effect on the tissue sample and the actual protocol for the experiment conducted. It also details and lists out the chemicals and the solutions used, the methodology used to obtain the tissue sample and their storage.

3.1 - Introduction:

This subsection details a set of experiments that were designed to increase the understanding about relaxed aortic stress and behavior of relaxed aortic tissue. Preliminary investigation on relaxed aortic tissue was done to confirm if vibration imposition did lead to a reduction in active forces. While previous literature did yield results for relaxation of ASMs and trachea, little is known about the aortic muscle tissue. These experiments have led to exploration of relaxed aortic muscle behavior and to develop the protocol used for this experiment.

The experimental study is based on a previous research approach to study ASMs, but has been modified to study the dynamics of aortic muscle tissue. While the basis for this study had researched ASMs and their relaxation in response to length and frequency, this study analysed just the effect of vibration on aortic muscle tissue and the response to it leading to development of better understanding of aortic muscle and management of cardiovascular diseases.

The study was carried out at Auckland University of Technology and all the set-up was acquired. The protocol for tissue handling, reference procedure and chemical solution preparation were developed. The set-up, control program and protocol are all discussed below.

3.2 - Equipment, procedures and protocol:

This section details out the description of the equipment, procedure and the program used in this research.

3.2.1- Materials:

The materials required for this study included Sodium Chloride (NaCl), Potassium Chloride (KCl), Potassium Phosphate (KH_2PO_4), Magnesium Sulfate ($MgSO_4$), Sodium Bicarbonate ($NAHCO_3$), Glucose, Calcium Chloride ($CaCl_2$) and MilliQ water primarily for the preparation of Krebs-Henseliet buffer solution. In addition to this a mixture of 95% O_2/ 5% CO_2 is required for bubbling through Krebs-Henseliet buffer solution. The aorta tissue is freshly obtained by slaughtering the animal.

3.2.2 – Tissue acquisition:

Prior to acquisition of aorta from rats, a physiological salt solution (Krebs-Henseliet buffer solution) (PSS, composition in mM: 118 NaCl, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25 $NAHCO_3$, 11 Glucose, 2.5 $CaCl_2$) was prepared in Millipore MilliQ 18 MΩ water. The solution was bubbled for at least 10 – 15 mins with 95% O_2/ 5% CO_2. This solution was prepared fresh every time prior to the experiment. The solution was kept at temperature of 37^0C and after checking the pH (7.35-7.45) was bubbled continuously. Another Krebs – Henseliet solution with slightly modified composition was prepared to induce contraction in the tissue (PSS, composition in mM: 53.93 NaCl, 60 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25 $NAHCO_3$, 11 Glucose, 2.5 $CaCl_2$) in Millipore MilliQ 18 MΩ water. This too was stored at 37^0C after bubbling with 95% O_2/ 5% CO_2 for 10 – 15 mins.

Fresh aorta was acquired by sacrificing grown male Wister Kyoto Rats at AUT by exposure to Carbon Dioxide (CO_2). The experiment had ethical approval from AUT. Each aorta was extracted from the animal within 10 mins of the slaughter. Connective tissue was removed and the aorta was flushed with Krebs-Henseliet solution to clean the excessive tissue, blood and other vessels. There was no need to transport the aorta as the animals were freshly slaughtered ay AUT's animal facility. All the work was carried out at controlled temperature of 24^0 C. Preliminary tests for the aorta's viability showed intense change in the viability within 72 hours with aorta becoming completely unresponsive to contracting and relaxing stimuli after 72 hours. The viability dropped continually when tested for 24 hours, 48 hours and 72 hours. Best viability was observed within 24 hours and hence all the experiments were conducted with fresh sample (<10 mins old) or with aorta stored at 4^0C in Krebs solution for less than 24 hour.

3.2.3 – Dissection:

A section of approximately 4-5 cm was dissected from the thoracic region and the abdominal region of the aorta. The aorta was then cleaned to remove any excessive connective tissue or blood till the sides of the aorta showed no excessive or loose appendages or bloody tissue (Fig 3.1). All of this was done in a tray filled with normal room temperature (37^0C) Krebs solution. The aorta then was cut using a sterile blade to yield aortic rings each about 2-4 mm in length. The aorta was not manipulated in any way to remove the internal epithelium or expose the smooth muscle cells. A silk thread (3/0 USP) with hooks was used to bind both the ends of the aorta which in turn was then suspended from the motor lever and base connector of the setup (Figure 4). Utmost care was taken not to impose any strain on the aortic ring sample.

Figure 3: Cleaned aorta sample.

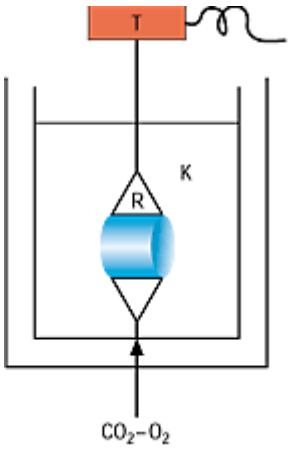

Figure 4: The tied aorta piece to the setup.

3.2.4 – Experimental Setup –

A tissue testing set up was assembled to test the effect of vibration imposition on the tissue sample. The set up and its components are explained in depth below.

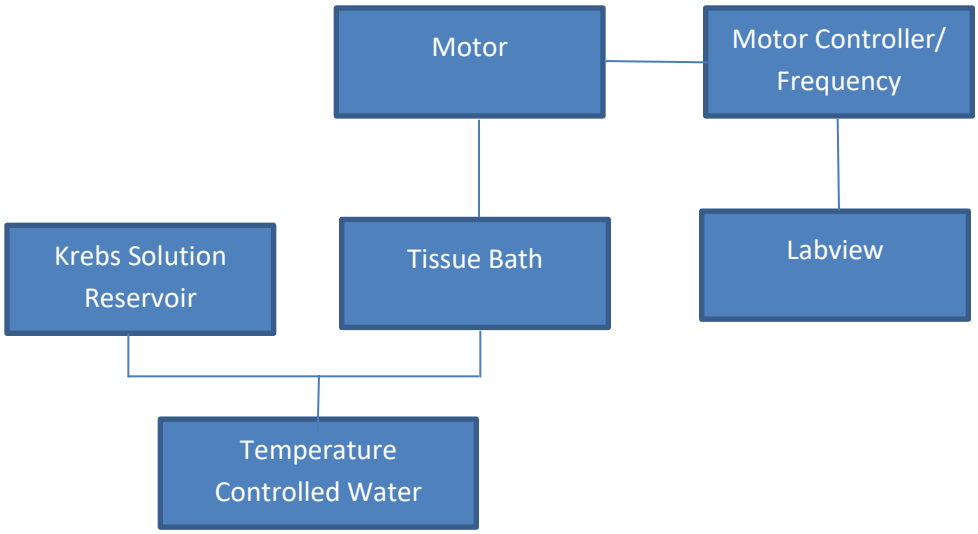

Figure 2: System diagram for tissue testing.

3.2.4.1 – Tissue Bath: This consists of a jacketed water reservoir (5 ml) that has inlet and outlet for temperature regulated water supply or other liquid supply. A bubbler rod which bubbles 95% O_2/ 5% CO_2 is inserted into the bottom connector and the bath has a bigger entry valve and a

smaller exit valve. The bath can be moved vertically and horizontally relative to the dual motor setup and tissue bottom clamp. The tissue is suspended vertically using silk thread, where one end is connected to the motor and another to a screw operated clamp.

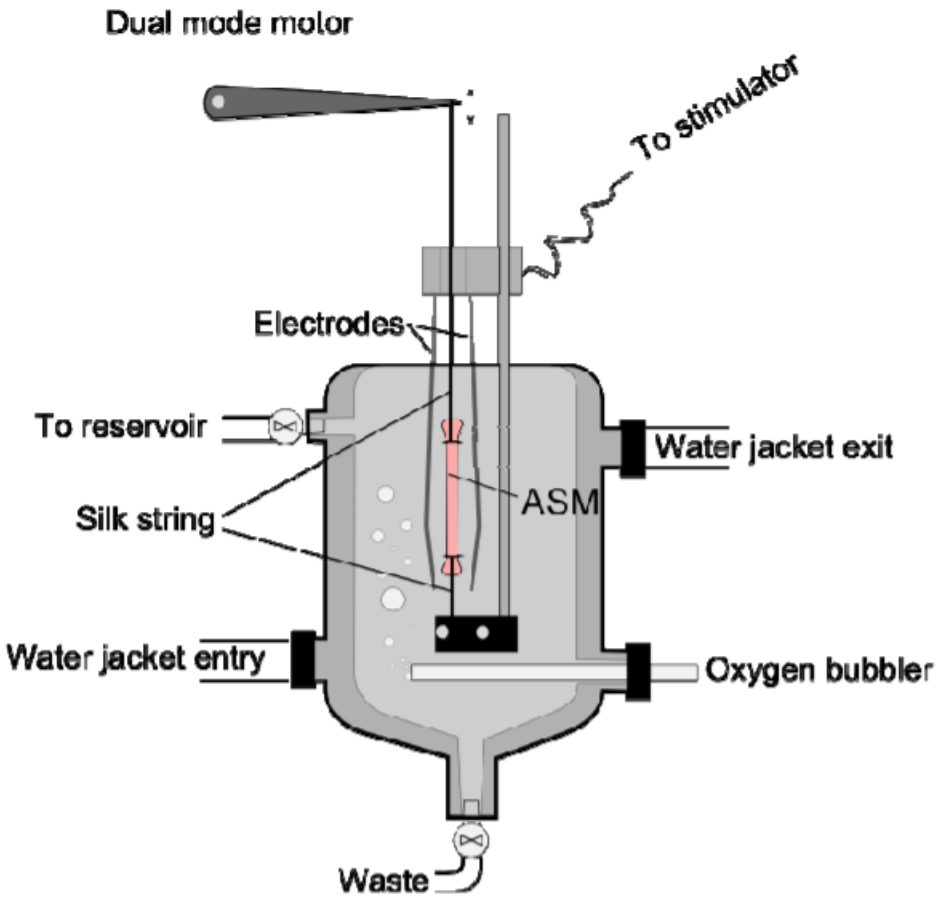

Figure 3: Tissue bath.

3.2.4.2 – Water Reservoir – A 1 Liter water jacketed reservoir is filled with Krebs and connected to the tissue bath. The reservoir generally has a heating coil used to heat the water to the required temperature. It is connected to the tissue bath and is regulated by a 2-way valve.

3.2.4.3 – Temperature control – A heating coil is used to maintain the water and the Krebs solution at the required temperature. For this study the general temperature is 37^0C and hence it is primarily used to heat up the water and the tissue bath along with the Krebs solution.

3.2.4.4 – Dual mode motor and controller – A Cambridge 300C dual mode motor and controller provide switchable length (0-3 mm range) and force (0-500 mN) control of the tissue by

analogue input signal or thru dial operation on the controller. The controller also provides an analogue output force and length signal with variable amplification (1-10x) with a maximum output range of 1 to 10 V on both channels with total ranges equal to the input range.

3.2.4.5 – Data Acquisition – The system was controlled through a Data Acquisition Card (NI 6024E) using National Labview (version 6.0)

3.2.5 – Reference Length Procedure –

Reference length procedure is used to minimize variability of tissue behavior between samples and to obtain maximum contraction and relaxation. There are different approaches of defining a reference length but none seem to be agreed upon unanimously. It is assumed that like ASMs, aortic tissue possesses a length range at which it can adapt to a stable optimal force generating capacity. However, since ASMs are strip and aortic tissue structure is a ring, our research focusses on using optimal length converted to optimal tension in Volts. Also, for this experiment a change of 0.4 V in tension translates to a change of about 1 mm and hence the tension range doesn't play a very important role. The tension range is most likely defined by the length of muscle tissue prior to slaughter, the age of the animal and the dissection and mounting storage. The purpose of a reference tension procedure is to find the present optimal length for maximum tension generation. The methodology used for finding the optimal tension and length has a strong influence on the optimal tension found. Several studies have shown changes in parameters such as stretch amplitude, contracting stimuli and timing can result in large difference in optimal length.(Du et al., 2007; Ijpma et al., 2010). A few of the commonly used procedures are described below,

3.2.5.1 – Single stretch from slack position – This procedure involves stretching the muscle by a small percentage of the slack strain under the assumption that this brings the tissue close to optimal length. However, studies have shown a clearly defined slack strain doesn't exist or is very difficult to establish (Oda, Taniguchi, & Yokoyama, 2001).

3.2.5.2 – Manually adjust to predetermined starting force – The length of the tissue is manipulated till a percentage of contractile force is reached. To correct for the offset in the contractile forces, the width or the estimated cross-sectional area of the tissue is used. However, if there is a difference in the starting length of the sample, their forces would be different.

3.2.5.3 – Direct search for optimal length – The most common approach to establishing an optimal length and in turn optimal tension is by conducting a manual search for it. A series of stretch, equilibration, contraction and relaxation cycle is applied and the reference length is defined as the one where contractile forces reduce from a cycle to the next.

From all the above-mentioned procedure, our research uses direct search for optimal length as it is most repeatable. Also, these methods do not guarantee establishing same reference length repeatedly. Another important advantage of the direct search method is it does not rely on slack length or variable of generating contractile force. Though this procedure is time consuming than others, if the time between contractions is defined and kept constant as is the tissue length, then the optimal length can be clearly defined. The only pitfall for this method is increasing the length to much or too little leading to either tissue damage or length adaptation during the procedure.

In this research a range of optimal length was found which translates to a range of optimal tension was found. The following procedure was used to develop the final range of tension.

1. After suspending the tissue in the tissue bath it can rest for 30-45 mins and equilibrate at around 1 nM of force.
2. The tissue is then subjected to the following cycles,
    - Stretch (approximately 5% of length)
    - Rest (5 mins) to allow force to stabilize
    - Changing the PSS to contracting Krebs Solution to establish current contractile force.
    - Rest (5 mins) to allow force to stabilize after contractile stimuli.
3. When the tissue showed lower contractile forces to the previous tension the tension range on the motor was noted.

The above procedure led to the discovery of optimal length between 0.480 and 0.520 V of tension and the rest of the protocol was followed within this range.

3.2.6 - Experimental protocol

This section lists out the actual steps that were followed once optimal tension and length was figured out.

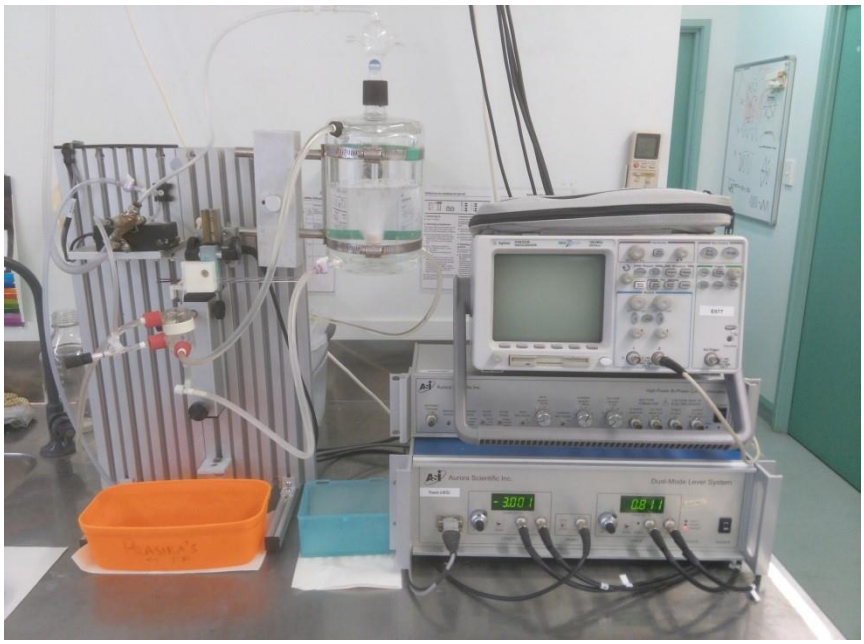

Figure 4: The entire experimental set up.

1. Aorta sections are obtained fresh at AUT by sacrificing an animal, immediately after an animal's death. The aorta sample are cleaned and placed in Krebs's physiological solution to maintain the tissue integrity.
2. The pH of Krebs's solution is maintained at around 7.4 ± 0.05 units with temperature of around 37^0C. It is bubbled with a mixture of 5% CO_2/O_2 mixture.
3. Fresh Krebs's solution is prepared every morning to store the tissues in.
4. Small rings of aorta are then cut from aortic rings to be used as sample strip for the experiment.
5. These aortic rings are then tided at both the end with suture. This is to attach the aorta ring with the dual mode lever system from Aurora Scientific, Canada, and suspend it in a small 5ml water bath with temperature maintained at 37^0c.
6. The lever system uses a stationary arm and a free moving arm to adjust the length of the tissue sample. This is essential when adjusting the length of the strip to find the reference length to be used.

7. Contractions and length are measured by the lever system in Volts. An increase in about 0.400 V in the length of the aorta strip corresponds to about 1mm length. This results in increase of contraction of about 0.070 to 0.100 units.

8. Once the reference length is found, it would be used and maintained for all strip samples. The reference length is used to calculate the actual relaxation obtained from the vibration imposition.

9. Contractions are induced in the aorta sample by using chemical stimuli of 60 mM Potassium Chloride (KCl). The amount of KCl or H_2O_2 is about 90ul which is added to 5ml of Krebs's solution in water bath.

10. Vibrations were then imposed in a fixed frequency range of (20-50)and amplitude of 1%. The amplitude of the vibration is generally calculated within the physiological limit of the tissue.

11. The duration of vibration imposition is generally measured in milliseconds and was kept constant at 200 milliseconds. Once the vibrations are imposed, a decrease in muscle active forces and subsequent decrease in contraction is seen.

12. The decrease in the active forces is measured and can be seen on the graph on a computer running the LabVIEW software attached to the dual mode lever system.

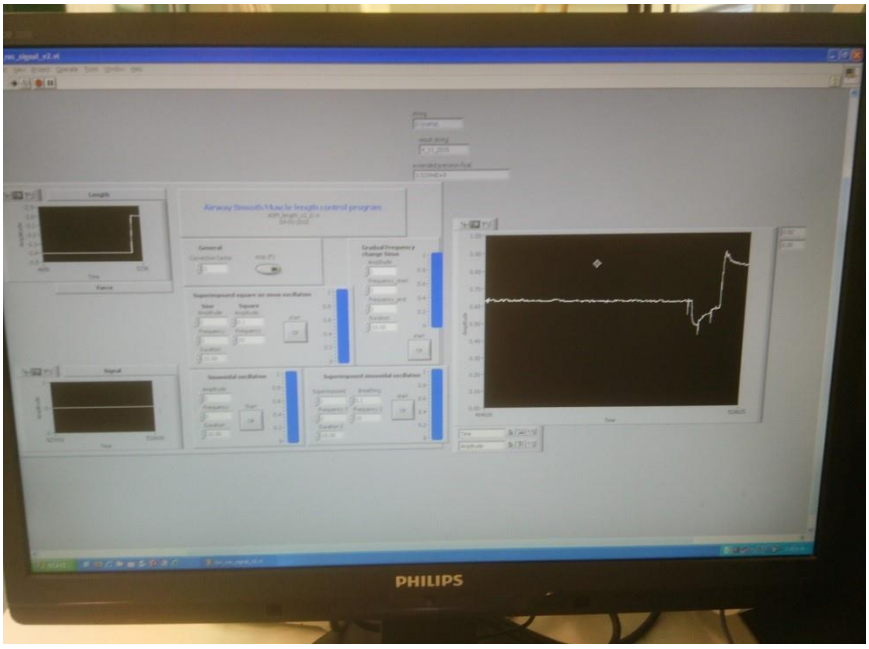

Figure 5: Labview software.

Chapter 4

Results and Discussion

Potassium Chloride (KCl) with a concentration of 60 mM induced contractions in the range of 0.170 – 0.230 V. The protocol uses tension in Volts to measure the stress induced on the tissue as reference length for the aorta tissue rings. Various frequencies of 20Hz, 30 Hz, 40 Hz and 50 Hz were used to induce relaxation in optimally contracted aorta rings. Sinusoidal vibrations were used since they are smooth and repetitive resulting in lower muscle fatigue and damage. The other factors used in sinusoidal vibrations such as the duration, the amplitude and angular phase all were maintained constant throughout the study. For this protocol, duration used was 200 milliseconds; amplitude was 1% of length and correction factor based on angular phase was 0.33 in accordance with previous literature.

The relaxation observed showed an upward curve with increasing frequency and hence it can be stated that various frequencies do have an effect in relaxation of the tissue sample. Beginning with 9-11% for 20 Hz, relaxation observed were 16% for 30 Hz, 21% for 40 Hz and 24% for 50 Hz on average (n=5 and relaxation =± 0.5%). These results indicate a clear rise in relaxation as there is a gradual increase in vibration frequency. A common observation was the decrease in relaxation percentage from one vibration imposition cycle to the next, leading to doubt of tissue fatigue. The actual observations are as follows,

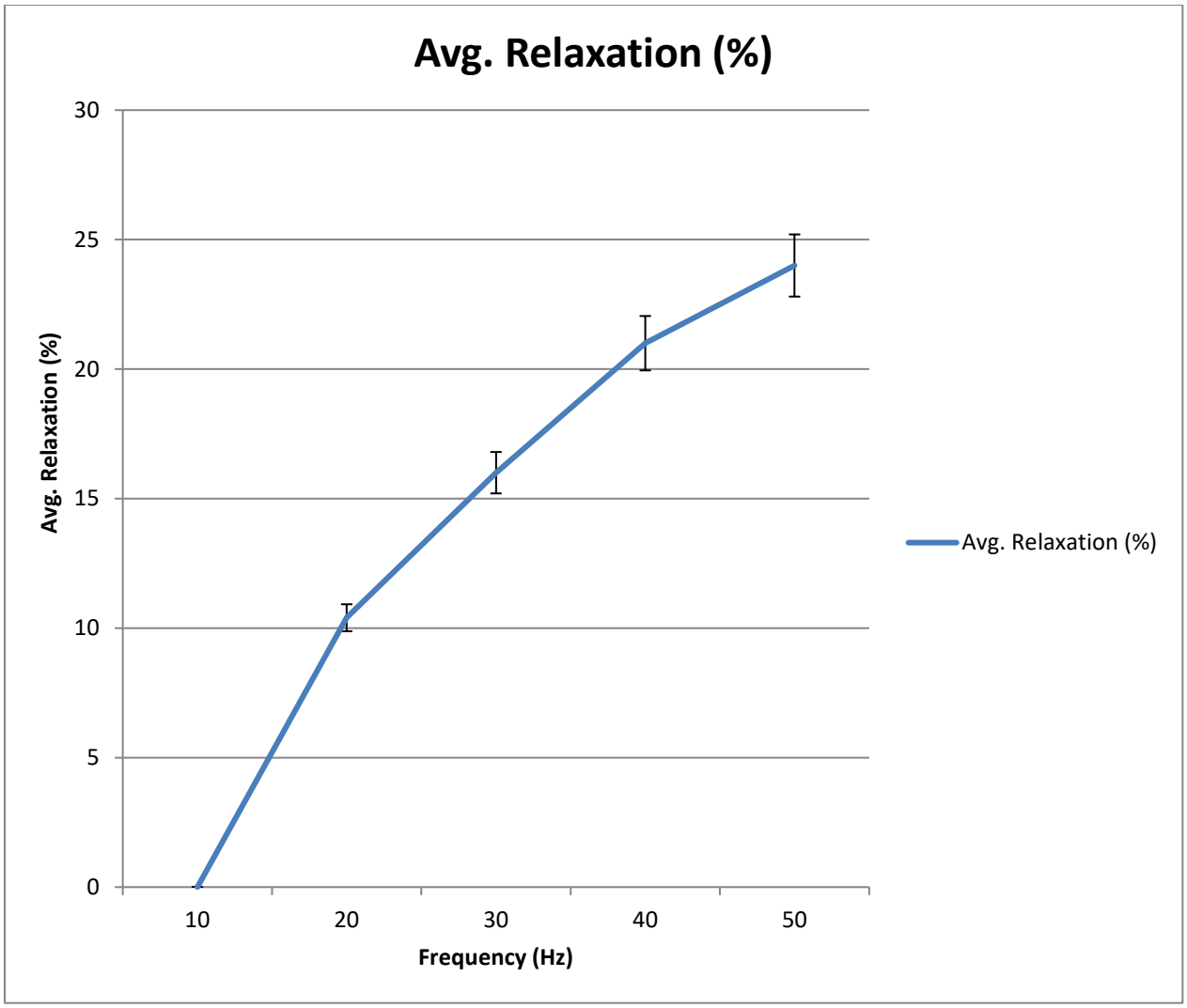

Figure 9: A Chart depicting the percentage of average relaxation seen as against the frequencies applied. 0% of relaxation can be seen at 10 HZ because the frequencies applied started from 20 Hz.

The first group was a control group to check for the optimal tension to provide maximum contracted tension, which was found in the ranges of 0.480 volts to 0.520 volts. Frequency of 20 Hz was applied just to check if relaxation was observed when the tissue sample was constricted optimally.

Set 1 Control Group, Frequency= 20 Hz, Duration = 200 ms, Correction factor = 0.33, Amplitude = 1%					
Starting Tension (V)	Length	Drained Tension (V)	Max Contractions (V)	Relaxed Tension (V)	Average Relaxation (%)
0.480	-4.706	0.443	0.480	0.380	21
0.490-0.491	-4.366	0.463	0.501	0.446	11
0.499-0.500	-4.076	0.478	0.541	0.507	7
0.510-0.513	-3.949	0.479	0.510	0.480	6
0.518-0.520	-4.076	0.478	0.541	0.507	7
Average Relaxation = 10.4%					

Table 1: Result table for percentage of relaxation seen across 5 cycles of 20hz vibration imposition for a control group.

Figure 10: Graph for the comparison of tension on the tissue during contraction (blue) vs. tension during relaxation (red) in volts for control group of 20hz (n=5).

The above graph shows the comparison between tension applied (in blue) and the reduction in tension (in red). Error bars are drawn on each observation with 5% percent error margin. Relaxation is greater when the frequency is applied initially and then plateaus off as vibrations are imposed in subsequent cycles. Overall the average relaxation is seen of 10.4% of the contraction tension in volts.

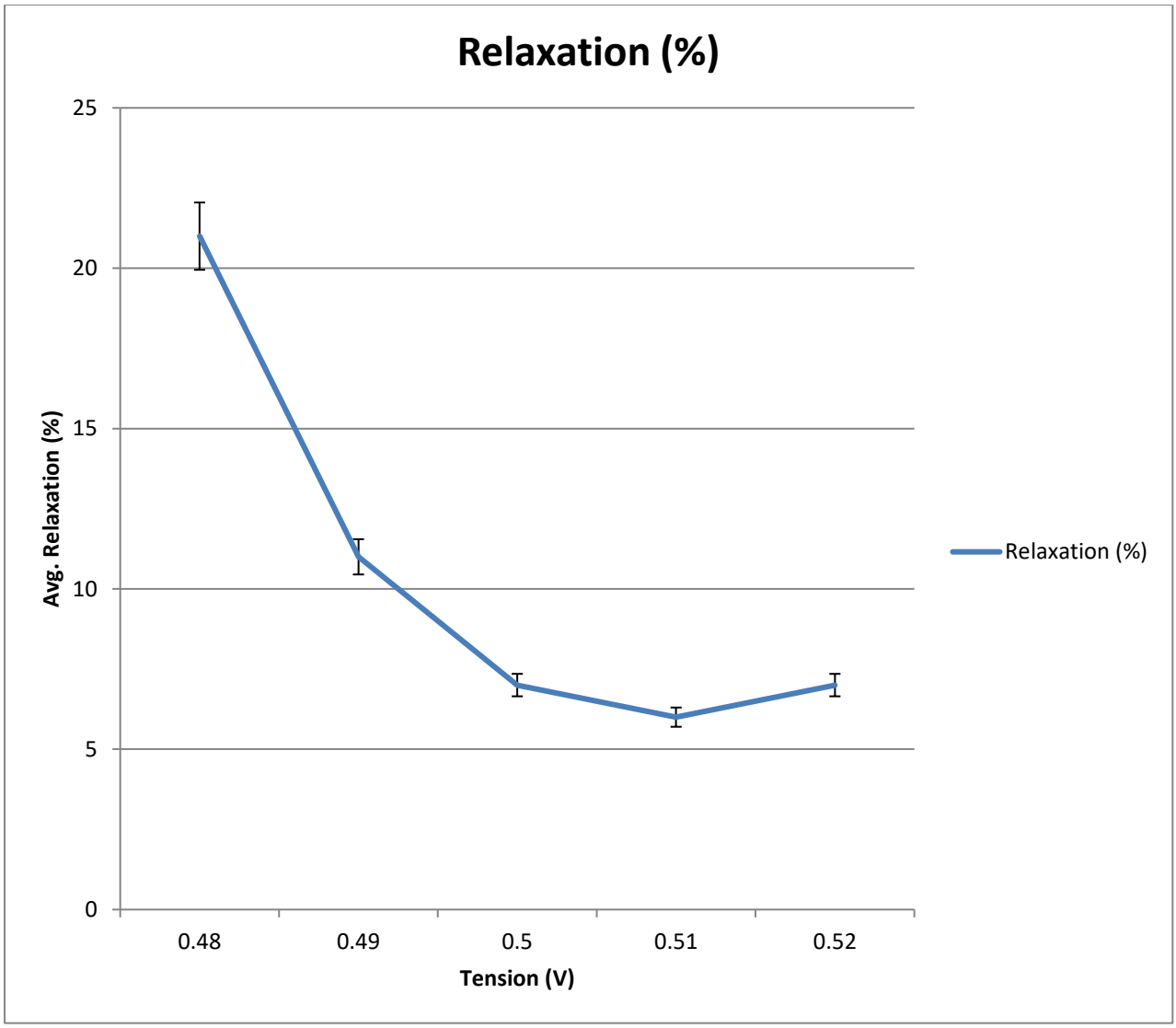

Figure 11: Average relaxation seen across 5 different cycles of 20 Hz frequency vibration (control group). The average relaxation seen is about 10.4% (n=5).

This is the actual population used to impose vibrations of 20 Hz and other parameters like amplitude and duration for the experiment were kept constant. The table below shows relaxation seen when multiple cycles of 20 Hz were applied on the tissue sample, similar percentage of relaxation was for the control group

Set 2, Frequency= 20 Hz, Duration = 200 ms, Correction factor = 0.33, Amplitude = 1%					
Starting Tension (V)	Length	Drained Tension (V)	Max Contractions (V)	Relaxed Tension (V)	Average Relaxation (%)
0.478-0.482	-5.049	0.398	0.487	0.436	11
0.491-0.495	-4.679	0.428	0.530	0.479	10
0.500-0.502	-4.471	0.440	0.576	0.526	9
0.509-0.511	-4.331	0.445	0.614	0.555	10
0.519-0.520	-4.221	0.463	0.638	0.593	7
Average Relaxation = 9.4%					

Table 2: Result table for percentage of relaxation seen across 5 cycles of 20hz vibration imposition.

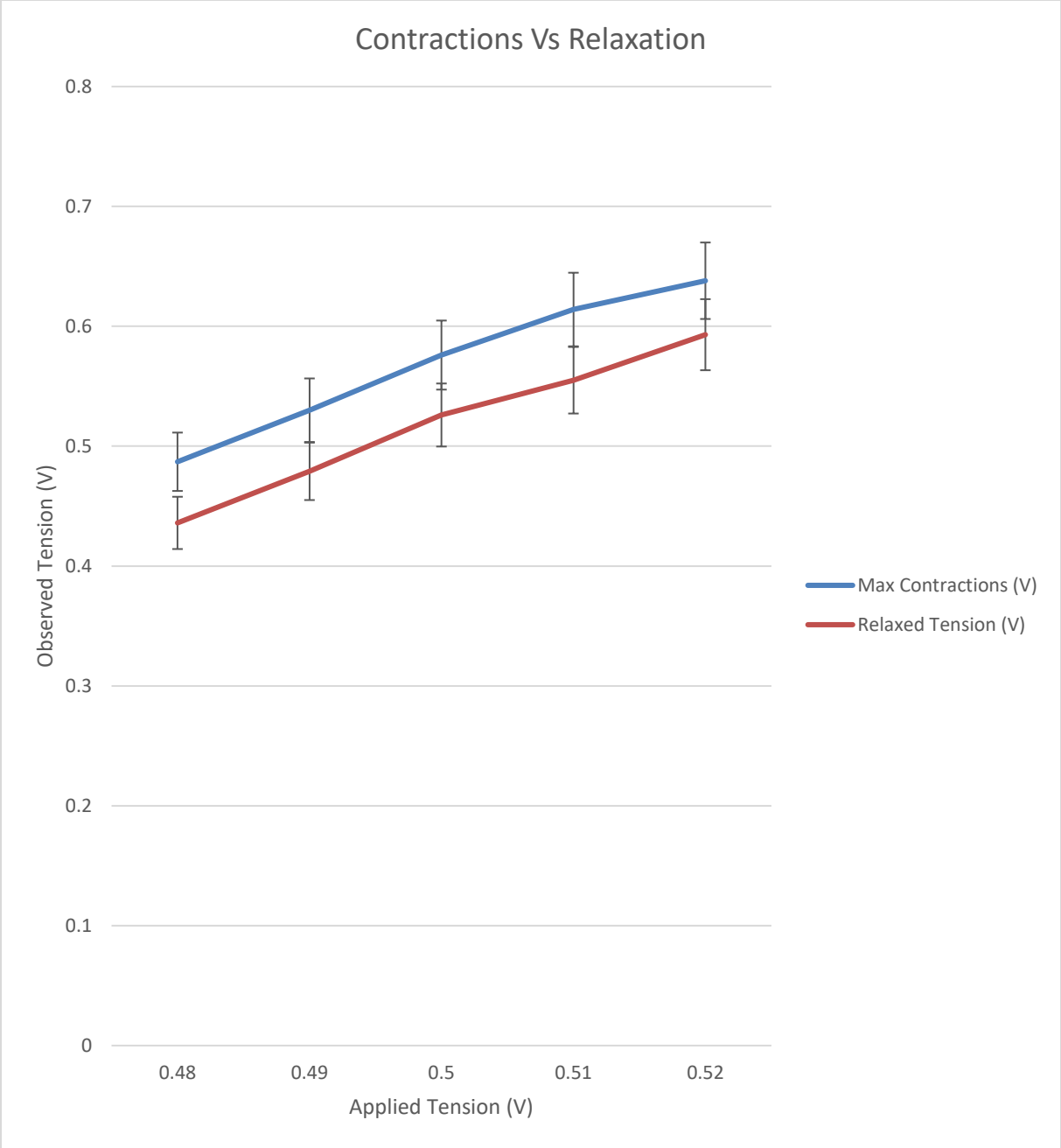

Figure 12: Graph representing comparison between tension during contraction (blue) and tension at relaxation (red) seen after imposing frequency of 20 Hz (n=5).

The above graph represents the difference between the tension during contraction (as seen in blue) vs relaxation (as seen in red). The general decrease in tension seems to be similar with relaxation increasing as the tension applied increases.

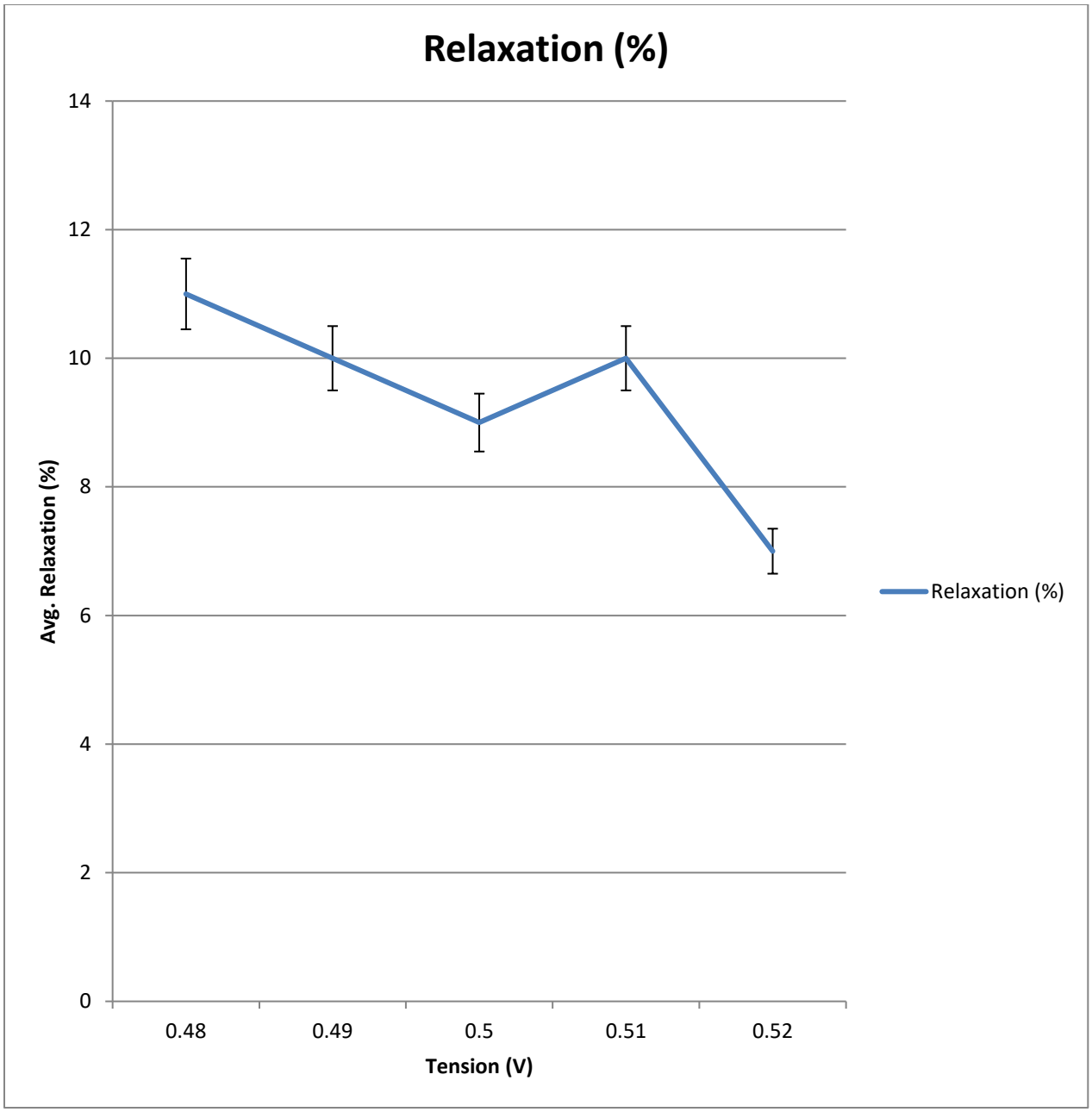

Figure 13: Average Relaxation for the 20 Hz frequency after each cycle of frequency imposition (n=5).

This group represents the population which was imposed with vibrations of 30 Hz frequency. As mentioned in the protocol before, all other parameters for the experiment were kept the same. The average relaxation for this group is seen at 16.4% of the contracted tension i.e. there is a difference of 16.4% on average between contracted tension and relaxed tension

Set 3, Frequency= 30 Hz, Duration = 200 ms, Correction factor = 0.33, Amplitude = 1%					
Starting Tension (V)	Length	Drained Tension (V)	Max Contractions (V)	Relaxed Tension (V)	Average Relaxation (%)
0.480-0.482	-4.875	0.460	0.492	0.377	24
0.489-0.490	-4.572	0.465	0.500	0.426	15
0.499-0.502	-4.541	0.450	0.501	0.448	11
0.510-0.511	-4.414	0.486	0.560	0.460	18
0.519-0.520	-4.193	0.475	0.520	0.448	14
Average Relaxation = 16.4%					

Table 3: Result table for percentage of relaxation seen across 5 cycles of 30hz vibration imposition

Figure 14: For 30 Hz frequency, the comparison between contractions (blue) and relaxation (red) is like the control group with relaxation plateauing of in the end (n=5).

The above graph represents the difference between the tension during contraction (as seen in blue) vs relaxation (as seen in red). The general decrease in tension seems to be similar with relaxation increasing as the tension applied increases and stays constant if the contraction tension doesn't change much.

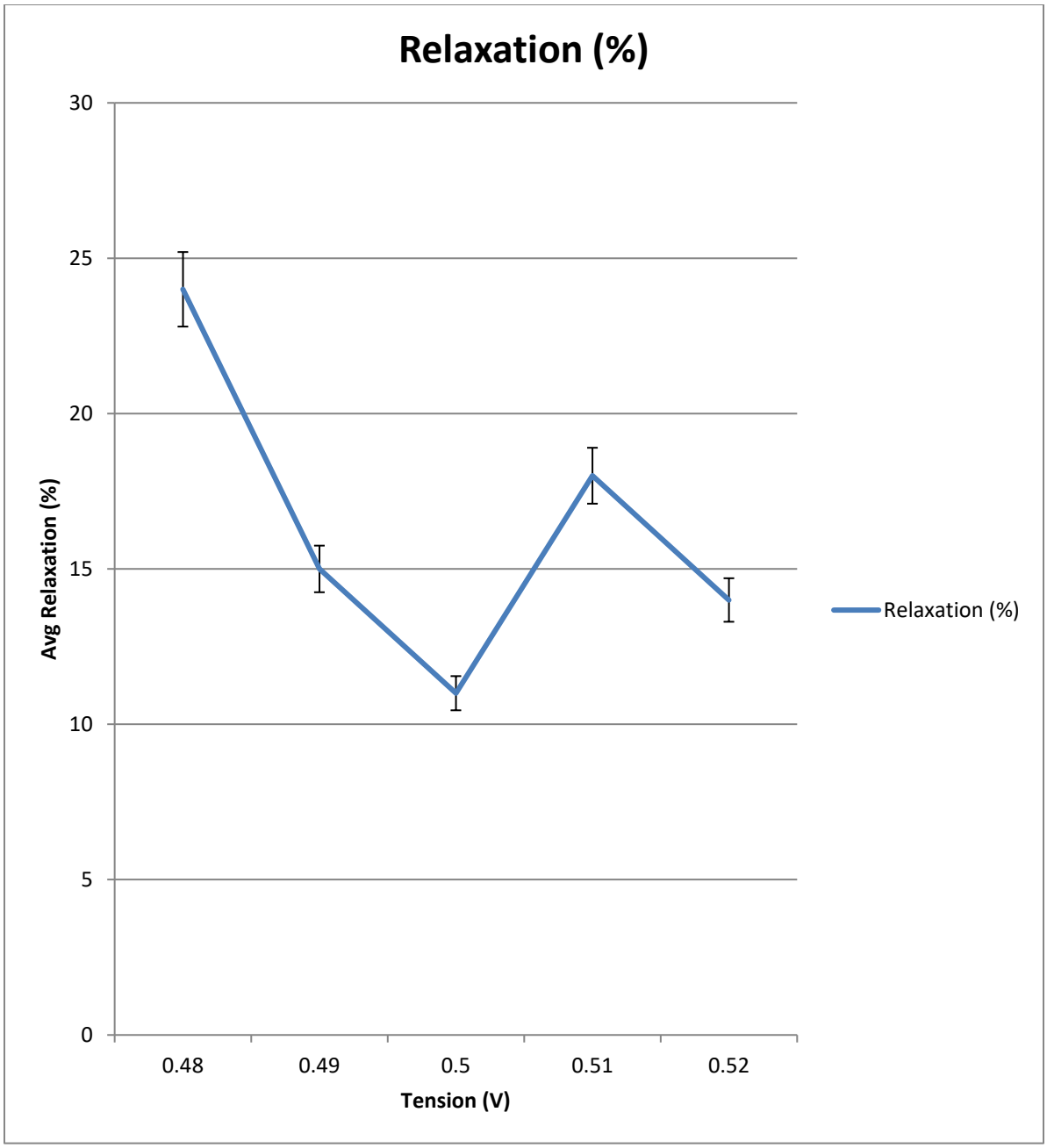

Figure 15: Average Relaxation for the 30 Hz frequency after each cycle of frequency imposition (n=5).

This group represents the population which was imposed with vibrations of 40 Hz frequency. As mentioned in the protocol before, all other parameters for the experiment were kept the same. The average relaxation for this group is seen at 21% of the contracted tension and further provides proof that as the frequency increases, the relaxation increases.

Set 4, Frequency= 40 Hz, Duration = 200 ms, Correction factor = 0.33, Amplitude = 1%					
Starting Tension (V)	Length	Drained Tension (V)	Max Contractions (V)	Relaxed Tension (V)	Average Relaxation (%)
0.480-0.483	-4.605	0.467	0.503	0.318	36
0.494-0.496	-3.925	0.480	0.524	0.400	24
0.502-0.504	-3.587	0.471	0.530	0.429	19
0.511-0.513	-3.339	0.487	0.542	0.475	13
0.521-0.523	-3.139	0.502	0.554	0.484	13
Average Relaxation = 21%					

Table 4: Result table for percentage of relaxation seen across 5 cycles of 40hz vibration imposition

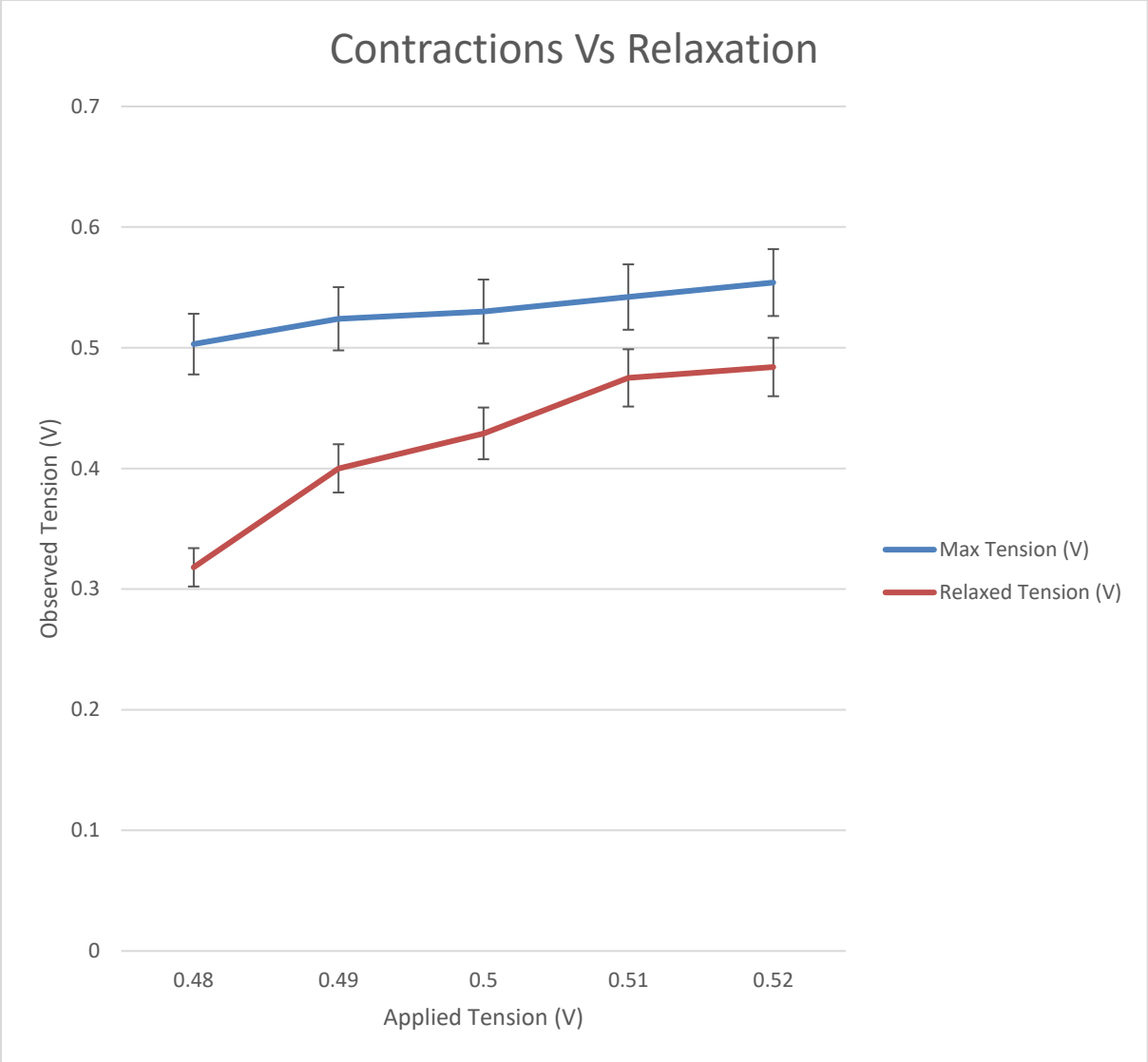

Figure 16: For 40 Hz frequency, the comparison between contractions (blue) and relaxation (red) shows the difference between contractile tension and relaxed tension decreases as cycles of 40 Hz frequency are completed (n=5).

The above graph represents the difference between the tension during contraction (as seen in blue) vs relaxation (as seen in red). The difference in contractile tension and relaxed tension decreases as multiple cycles of 40 Hz are applied on the tissue sample.

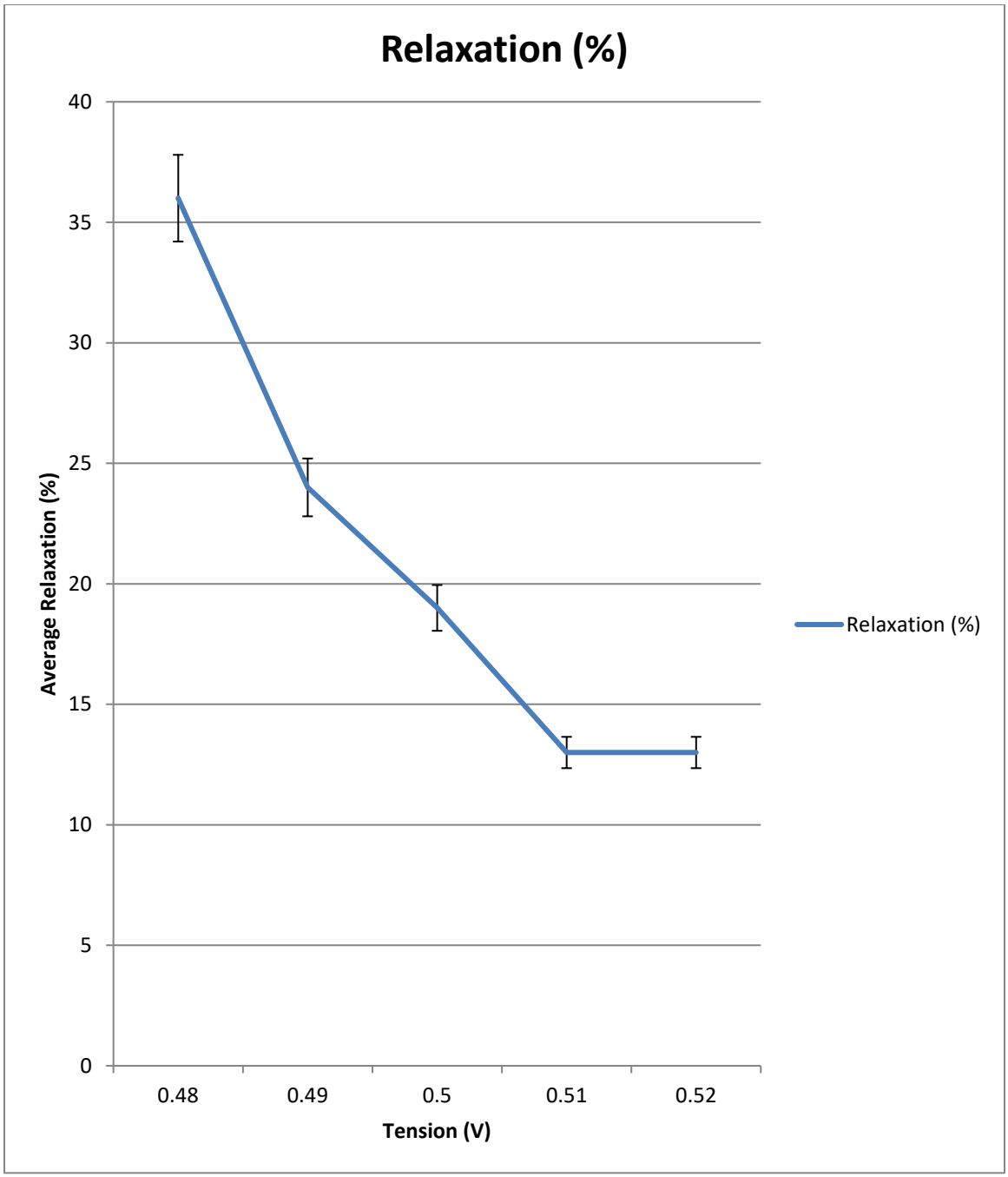

Figure 17: Average Relaxation for the 40 Hz frequency after each cycle of frequency imposition (n=5).

The final group of the study represents the population which was imposed with vibrations of 50 Hz frequency. As mentioned in the protocol before, all other parameters for the experiment were kept the same. The average relaxation for this group is seen at 24% of the contracted tension and although the difference is only 3% when compared with 40 Hz group, still it provides proof to the hypothesis that as frequency increases relaxation seen increases as well.

Set 1, Frequency= 50 Hz, Duration = 200 ms, Correction factor = 0.33, Amplitude = 1%					
Starting Tension (V)	Length	Drained Tension (V)	Max Contractions (V)	Relaxed Tension (V)	Average Relaxation (%)
0.488-0.489	-1.661	0.478	0.509	0.290	46
0.499-0.500	-0.824	0.479	0.540	0.351	35
0.509-0.511	-0.301	0.492	0.561	0.440	22
0.517-0.519	-1.219	0.508	0.660	0.597	11
0.527-0.528	-1.138	0.514	0.643	0.615	6
Average Relaxation = 24%					

Table 5: Result table for percentage of relaxation seen across 5 cycles of 50hz vibration imposition.

Figure 18: For 40 Hz frequency, the comparison between contractions (blue) and relaxation (red) shows the difference between contractile tension and relaxed tension decreases as cycles of 50 Hz frequency are completed, almost meeting at the end of fifth cycle (n=5).

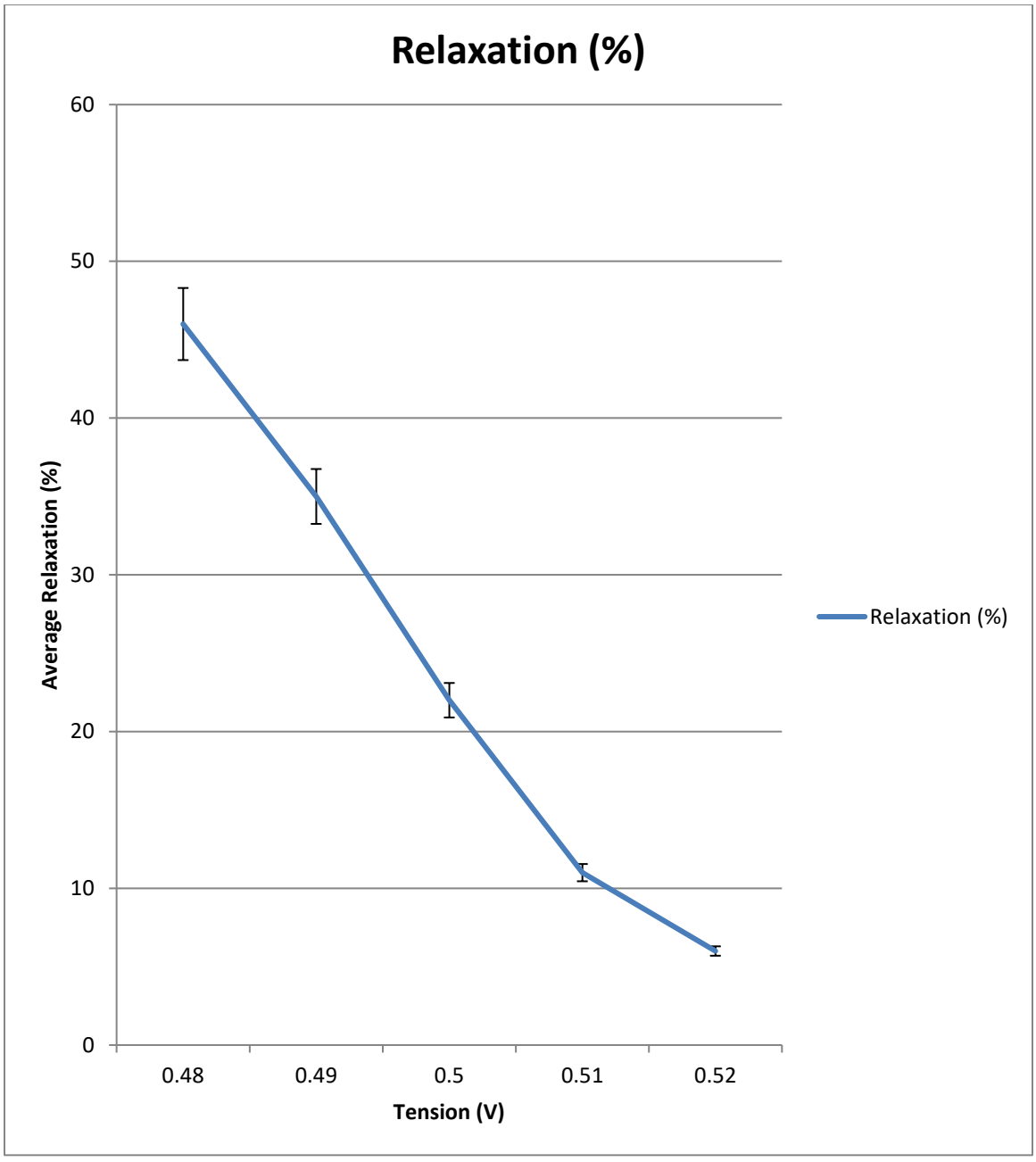

Figure 19: Average Relaxation for the 50 Hz frequency after each cycle of frequency imposition (n=5)

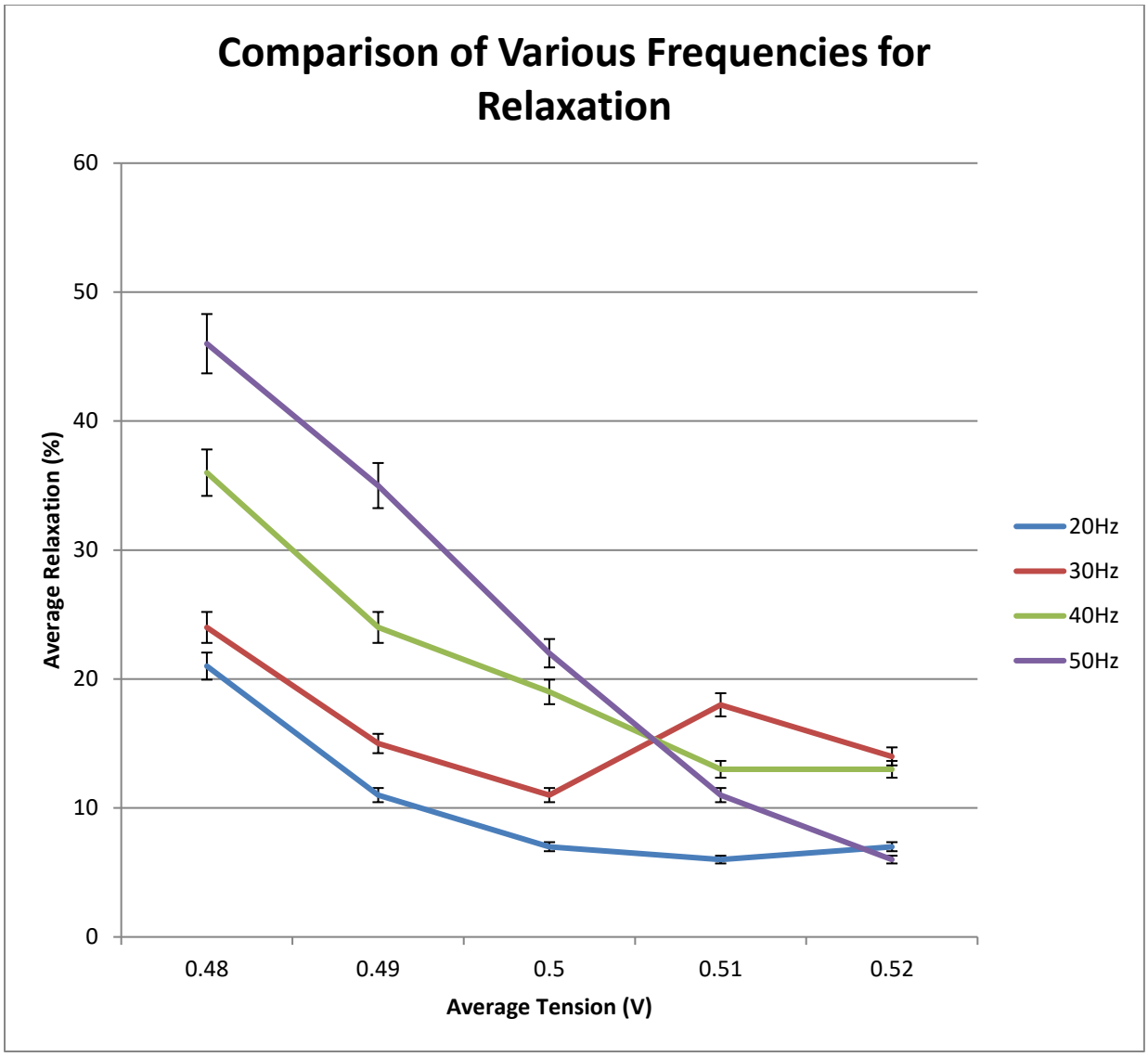

Figure 20: The graph comparing average relaxation percentage after imposition of 20, 30, 40 and 50 Hz frequencies (n=5).

4.1 - Statistical Analysis:

4.1.1 - ANOVA:

ANOVA or Analysis of Variance is commonly used technique to investigate data by comparing the means of subset of data. We have performed a one-way ANOVA, which is an extended version of two-sample t-test to compare the relaxation and contractions seen in the muscle tissue. In one-way ANOVA, the data is sub divided into groups based on just one factor which classifies the data, and the terminology used to refer to this factor is treatment, although it might not mean the same in literal sense. ANOVA also investigates if whether the variation that's seen in the measurement taken on the individual components of the data set can be explained by the grouping introduced. ANOVA, like the other statistical tests has a set of two hypotheses defined commonly referred to as "Null Hypothesis" and "Alternative Hypothesis". These are exclusive to each other, meaning if one of them holds true, the other must be false and vice versa. The two hypotheses can never be true together nor can they be false together.

ANOVA like the t-test depends on the p-value and the significance level (α) to choose between the hypothesis that are claimed by the data. For ANOVA, if the P-value $\leq \alpha$, then null hypothesis is rejected i.e. alternative hypothesis is accepted and conclusion is drawn as to not all population means are equal and the grouping factor has significant effect. If the P-value $\geq \alpha$, then we accept null hypothesis and conclusion drawn is all population means are equal and the grouping factor has no significant effect on the population.

For our study, the two variables for ANOVA are the contraction observed on the tissue and the relaxation seen. These two variables are selected to check their dependence in terms of relaxation observed after predefined frequencies are applied. The two hypotheses are as follows,

1. Null Hypothesis: The relaxation seen for the respective frequencies have same mean and hence, frequencies have no effect on relaxation
2. Alternative Hypothesis: The relaxation seen for the respective frequencies have different mean and hence relaxation and frequencies imposed are significantly related to each other.

Frequency (Hz)	P-value	Significance Level (α)	Hypothesis Accepted
20	Lesser than α	0.05	Alternative hypothesis
20	Lesser than α	0.05	Alternative hypothesis
30	Greater than α	0.05	Null hypothesis
40	Lesser than α	0.05	Alternative hypothesis
50	Lesser than α	0.05	Alternative hypothesis

Table 6: Result table for ANOVA for various frequency groups

As seen from the result table above, for the 20 Hz, 40 Hz and 50 Hz frequency, we accept the alternative hypothesis as P-value is lower than the significance level of 0.05 and hence it indicates that for the said frequencies relaxation depends on the frequencies applied and there is a significant relaxation between the two. However, for the 30 Hz frequency, p-value is greater than significance level and hence we should accept the null hypothesis which states the frequency (30 Hz) has no effect on relaxation. This contradicts the other findings and can be due to low number of sample considered for this experiment (n =5). Hence a one sample t-test is carried to compare the mean with the population and draw a conclusion as to why this has happened.

4.1.2 One Sample t-test:

One sample t-test calculates the difference between sample mean and the hypothesized mean relative to the variability of the sample. Usually, the larger the difference and the smaller the variability in your sample, greater the chance that the population mean differ significantly from the hypothesized mean. We would be using the one sample t-test to test the group which was imposed with vibrations of 30 Hz frequency. We will compare individual samples to the group mean with individual values and try to check for significant difference, thus giving us the value which caused the ANOVA test for 30 Hz to return null hypothesis. Hence, we are using one sample t-test to check whether the group mean for 30 Hz differs from individual values in the 30 HZ group.

One sample t-test is used to compare a known mean value with the entire population values and conclude based on comparison of the P-value with the level of significance (α). Like ANOVA if the p-value < significance = reject null hypothesis and accept alternative hypothesis and if p-value > significance = accept null hypothesis and reject alternative hypothesis. For this study the two hypotheses are as follows,

1. Null Hypothesis = the mean is equal to the true mean of the population i.e. there is no significance between the two values
2. Alternative hypothesis = the mean is not equal to the true mean of the population i.e. there is a significance between the two values.

The result from the t-test are shown below in the table.

Frequency (Hz)	Mean Value	P-Value	Significance Level (α)	Hypothesis Accepted
30	0.377	Lesser than α	0.05	Null
30	0.426	Greater than α	0.05	Alternative
30	0.448	Greater than α	0.05	Alternative
30	0.460	Greater than α	0.05	Alternative
30	0.448	Greater than α	0.05	Alternative

Table 7: Result table for one sample t-test (30hz frequency)

From the above table, all the values are significant except the first and hence, it can be said that with an increase in population size (n > 5), this value would change and would attend significance as well.

Chapter 5

Discussion and Future Work

From the experiments and the result, vibrations when imposed on aortic tissue help in reducing tension and active muscle forces. There is also an immense scope for future work and research based on this research. The most important point to notice in my research would be the increase in average relaxation across the tissue sample with the increase in frequency. From this we can say the relaxation would increase continually till a frequency where the maximum relaxation can be around 30% of the contractile forces would be achieved. This can, as described before, lead to a development of a localized form of treatment. It can probably replace the traditional methods of treatment of cardiovascular diseases such as oral drugs and surgery. The challenge here though would be maintaining the level of relaxation over multiple cycles as it can be seen from the results that the average relaxation percentage and tension decreases from one cycle to next for any given frequency. The plateau seen in the graphs of relaxation also indicates that the relaxation seen by the tissue sample reaches a peak maximum and can't be furthered. This aspect of the imposition of frequency on the tissue sample needs to be addressed.

Another important factor which my research validates is that although ASMs and aorta are structurally and functionally different, they relax under vibration imposition. This leads to furthering of this research for other tissues such as the carotid artery and the jugular vein which have similar structure to aorta. The key aspect as to why aorta and ASMs relax is because they are essentially made up of smooth muscle cells and their biomechanical properties are governed by these smooth muscle cells. When the hypothesis of this experiment is tested on tissue or muscle samples which have smooth muscle cell structure, it would further the understanding of smooth muscle and their relaxation. The finding of this research can also be applied on the cardiovascular smooth muscle cells such as those of the heart and see if there is a significant relaxation in them as well.

My research only studied the effect of a chemical stimuli – Potassium Chloride (KCl) on the tissue sample. KCl contracts a muscle tissue by acting on its Potassium/Calcium (K^+/Ca^+) channels (Aidley, 1978; Ohlstein & Douglas, 1993; Reynolds et al., 2004). It leads to accumulation

of potassium inside the cell and blocks the normal functioning of the cell. Other chemical stimuli such as Hydrogen Peroxide (H_2O_2) and Calcium Chloride ($CaCl_2$) induce contractions in muscle by different mechanisms such as by generating Reactive Oxygen Species (ROS) (commonly called as free radicals) or by unbalancing the level of ions in the cell respectively. Further researches in muscle contraction can focus on either of the two methods of generating contractions.

Another important aspect to consider is the concentration of chemical stimuli used. In my research, only 60 mM of KCl was used. As mentioned in the literature review, various concentrations of KCl have varied responses for contraction from the muscle tissue. This can be extrapolated to say that even for various contractions elicited by use of H_2O_2 and $CaCl_2$ the would be different and the amount of contractile force would change as well.

The final gap for further studies that this research has left is development of mathematical model for aortic tissue relaxation. This research has completed a statistical analysis of the data and found significant results in respect to rat aorta, but this information is incomplete and applicable only to a specific tissue sample of a specific species. If a comprehensive mathematical model is developed, it might be able to fit the entire concept of relaxation of tissue samples from various species and provide us with a better picture of the entire result. However, because of the time constrain and development of a novel methodology, developing a mathematical model to fit the results has not been possible. Also, the number of sample population ($n = 5$) would be too less to develop a mathematical model and would need an increase in the data size. Other parameters if any, like different contraction mechanism or the blood pressure frequency would also need to be included in the mathematical model.

To summarize, I would say this research is combination of a through literature review, development of novel methodology with its implementation and future scope in this study. The literature review although is not extensive, quite rare and old yields certain important pathways to follow for goals and has been instrumental in shaping the outcome of this research. The field of muscle relaxation using vibrations is an ocean of knowledge that has been unexplored and hides lot of ideas and contributions. To cover these ideas and pathways would have required a lot of time and analysis and hence, we had to stick to the most important and related topic which was the proving that aortic muscle relax under vibrations and that vibrations relax various tissues. Right from hypothesizing that aortic smooth muscle would relax under vibrations like what's seen in

ASMs to developing methodology for optimal length and tension and the experimental protocol, this research has tried to address several issues associated with muscle contraction and relaxation. The idea of completely contracting the muscle sample by changing the PSS to a contracting PSS and then exposing this setup to vibration was instrumental in determining the contractile force and relaxation.

This research and the result derived after the experimentation has left us with more gaps to be filled in the future, some of which have been explained above, and advancements to be made in this field. Although the data and methods available for research in this field is rare and old, my research opens new dimensions for future work to take place.

References:

Aidley, D. J. (1978). *The physiology of excitable cells*: Cambridge; New York : Cambridge University Press, 1978. 2nd ed.

Angus, J. A., Cocks, T. M., McPherson, G. A., & Broughton, A. (1991). The acetylcholine paradox: a constrictor of human small coronary arteries even in the presence of endothelium. *Clinical and experimental pharmacology and physiology, 18*(1), 33-36.

Burns, G. P., & Gibson, G. J. (1998). Airway hyperresponsiveness in asthma. Not just a problem of smooth muscle relaxation with inspiration. *Am J Respir Crit Care Med, 158*(1), 203-206. doi:10.1164/ajrccm.158.1.9707125

Cliff, W. J. (1967). The aortic tunica media in growing rats studied with the electron microscope. *Lab Invest, 17*(6), 599-615.

Cui, D. D. (2011). *Atlas of histology : with functional and clinical correlations*: Philadelphia : Wolters Kluwer Health/Lippincott Williams & Wilkins, [2011] First edition.

Dowe, D. A., Fioranelli, M., & Pavone, P. (2013). *Imaging coronary arteries*: Milan ; Heidelberg : Springer, [2013].

Drake, R. L. P. D., Vogl, W., Mitchell, A. W. M., Gray, H., & Based on : Gray, H. A. (2010). *Gray's anatomy for students*: Philadelphia, Pa. : Churchill Livingstone/Elsevier, [2010] Second edition.

Du, Y., Al-Jumaily, A. M., & Shukla, H. (2007). *Smooth muscle stiffness variation due to external longitudinal oscillations*.

Fabry, B., & Fredberg, J. J. (2003). Remodeling of the airway smooth muscle cell: are we built of glass? *Respir Physiol Neurobiol, 137*(2-3), 109-124.

Fiore, M. S. H. d., & Schmidt, I. G. (1974). *Atlas of human histology*: Philadelphia : Lea & Febiger, 1974.Fourth edition / Revised by Ida G. Schmidt.

Furchgott, R. F., & Zawadzki, J. V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature, 288*(5789), 373-376.

Gerthoffer, W. T. (2005). Actin cytoskeletal dynamics in smooth muscle contraction. *Can J Physiol Pharmacol, 83*(10), 851-856. doi:10.1139/y05-088

Gibbs, C. L., & Chapman, J. B. (1985). Cardiac mechanics and energetics: chemomechanical transduction in cardiac muscle. *American Journal of Physiology-Heart and Circulatory Physiology, 249*(2), H199-H206.

Grotenhuis, H. B., & de Roos, A. (2011). Structure and function of the aorta in inherited and congenital heart disease and the role of MRI. *Heart, 97*(1), 66-74. doi:10.1136/hrt.2010.198713

Gunst, S. J., & Wu, M.-F. (2001). Selected Contribution: Plasticity of airway smooth muscle stiffness and extensibility: role of length-adaptive mechanisms. *Journal of Applied Physiology, 90*(2), 741.

Guyton, A. C., & Hall, J. E. (2000). *Textbook of medical physiology*: Philadelphia, Pa. ; London : W. B. Saunders, [2000] Tenth edition.

Ijpma, G., Al-Jumaily, A., Cairns, S., & Sieck, G. (2010). *Airway smooth muscle dynamics : a thesis submitted to Auckland University of Technology in fulfilment of the requirements for the degree of Doctor of Philosophy*.

Lang, E. V. (1997). Functional Abnormalities of the Aorta. *Journal of Vascular and Interventional Radiology, 3*(8), 382.

Lever, J. D., Ahmed, M., & Irvine, G. (1965). Neuromuscular and intercellular relationships in the coronary arterioles. A morphological and quantitative study by light and electron microscopy. *J Anat, 99*(Pt 4), 829-840.

Martin, E. (2015). *cardiovascular system circulatory system*: Oxford University Press.

Matthews, M. A., & Gardner, D. L. (1966). The fine structure of the mesenteric arteries of the rat. *Angiology, 17*(12), 902-931. doi:10.1177/000331976601701203

McManaman, J. L., Reyland, M. E., & Thrower, E. C. (2006). Secretion and fluid transport mechanisms in the mammary gland: comparisons with the exocrine pancreas and the salivary gland. *J Mammary Gland Biol Neoplasia, 11*(3-4), 249-268. doi:10.1007/s10911-006-9031-3

Netter, F. H. (2014). *Atlas of human anatomy*: Philadelphia, PA : Saunders/Elsevier, [2014] Sixth edition.

Nichols, W., O'Rourke, M., & Vlachopoulos, C. (2011). *McDonald's blood flow in arteries: theoretical, experimental and clinical principles*: CRC Press.

Oda, A., Taniguchi, T., & Yokoyama, M. (2001). Leptin stimulates rat aortic smooth muscle cell proliferation and migration. *Kobe Journal of Medical Sciences, 47*(3), 141-150.

Ohlstein, E. H., & Douglas, S. A. (1993). Endothelin-1 modulates vascular smooth muscle structure and vasomotion: Implications in cardiovascular pathology. *Drug Development Research, 29*(2), 108-128. doi:10.1002/ddr.430290207

Pérez-Vizcaíno, F., Fernández del Pozo, B., Zaragozá, F., & Tamargo, J. (1994). Voltage- and time-dependent inhibitory effects on rat aortic and porcine coronary artery contraction induced by propafenone and quinidine. *British Journal of Pharmacology, 113*(4), 1281-1288.

Poole, K. J. V., Lorenz, M., Evans, G., Rosenbaum, G., Pirani, A., Craig, R., . . . Holmes, K. C. (2006). A comparison of muscle thin filament models obtained from electron microscopy reconstructions and low-angle X-ray fibre diagrams from non-overlap muscle. *Journal of Structural Biology, 155*(2), 273-284. doi:http://dx.doi.org/10.1016/j.jsb.2006.02.020

Rall, J. A., & Woledge, R. C. (1990). Influence of temperature on mechanics and energetics of muscle contraction. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 259*(2), R197-R203.

Reynolds, J. L., Joannides, A. J., Skepper, J. N., McNair, R., Schurgers, L. J., Proudfoot, D., . . . Shanahan, C. M. (2004). Human vascular smooth muscle cells undergo vesicle-mediated calcification in response to changes in extracellular calcium and phosphate concentrations: a potential mechanism for accelerated vascular calcification in ESRD. *J Am Soc Nephrol, 15*(11), 2857-2867. doi:10.1097/01.asn.0000141960.01035.28

Rhoades, R., & Bell, D. R. (2009). *Medical physiology : principles for clinical medicine*: Philadelphia : Lippincott Williams & Wilkins, [2009] Third edition.

Rhodin, J. A. (1962). Fine structure of vascular walls in mammals with special reference to smooth muscle component. *Physiol Rev Suppl, 5*, 48-87.

Silverthorn, D. U., Johnson, B. R., Ober, W. C., Garrison, C. W., & Silverthorn, A. C. (2013). *Human physiology : an integrated approach*: Boston : Pearson Education, [2013] Sixth edition.

Somlyo, A. P., & Somlyo, A. V. (1968). Vascular smooth muscle. I. Normal structure, pathology, biochemistry, and biophysics. *Pharmacol Rev, 20*(4), 197-272.

Stöllberger, C. (2014). *Lifeblood flow: The circulatory systems* (Vol. 9783709115596): Springer-Verlag Wien.

Trepat, X., Deng, L., An, S. S., Navajas, D., Tschumperlin, D. J., Gerthoffer, W. T., . . . Fredberg, J. J. (2007). Universal physical responses to stretch in the living cell. *Nature, 447*(7144), 592-595. doi:http://www.nature.com/nature/journal/v447/n7144/suppinfo/nature05824_S1.html

Trepat, X., Grabulosa, M., Puig, F., Maksym, G. N., Navajas, D., & Farre, R. (2004). Viscoelasticity of human alveolar epithelial cells subjected to stretch. *Am J Physiol Lung Cell Mol Physiol, 287*(5), L1025-1034. doi:10.1152/ajplung.00077.2004

Wang, L., & Pare, P. D. (2003). Deep inspiration and airway smooth muscle adaptation to length change. *Respir Physiol Neurobiol, 137*(2-3), 169-178.

Widmaier, E. P., Raff, H., & Strang, K. T. (2008). *Vander's human physiology : the mechanisms of body function*: Boston : McGraw-Hill Higher Education, [2008] Eleventh edition.

Zoghbi, M. E., Woodhead, J. L., Moss, R. L., & Craig, R. (2008). Three-dimensional structure of vertebrate cardiac muscle myosin filaments. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(7), 2386-2390. doi:10.1073/pnas.0708912105