A COMPUTATIONAL APPROACH TO STUDY THE EFFECT OF MULTIPLE LYMPHANGION COORDINATION ON LYMPH FLOW

A Thesis

by

ARUN MADABUSHI VENUGOPAL

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2004

Major Subject: Biomedical Engineering

A COMPUTATIONAL APPROACH TO STUDY THE EFFECT OF MULTIPLE LYMPHANGION COORDINATION ON LYMPH FLOW

A Thesis

by

ARUN MADABUSHI VENUGOPAL

Submitted to Texas A&M University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Approved as to style and content by:

Christopher M. Quick (Chair of Committee)	John C. Criscione (Member)
Randolph H. Stewart (Member)	David C. Zawieja (Member)

William A. Hyman
(Head of Department)

August 2004

Major Subject: Biomedical Engineering

ABSTRACT

A Computational Approach to Study the Effect of Multiple Lymphangion Coordination on Lymph Flow. (August 2004)

Arun MadabushiVenugopal, B.E., Madras University, Chennai, India

Chairs of Advisory Committee: Dr. Christopher M. Quick

The lymphatic system acts to return fluid from the interstitial space back into the blood circulation. In normal conditions, lymphangions, the segment of lymphatic vessel in between valves, cyclically contract and can pump lymph from low pressure tissues to the higher-pressure veins of the neck. With edema, however, this pressure gradient can reverse, and the role of contraction is less clear. Like ventricles, lymphangions are sensitive to both preload and afterload. Unlike ventricles, lymphangions are arranged in series, so that the outlet pressure of one lymphangion becomes the inlet pressure of another. Anything that alters the relative timing and frequency of adjacent lymphangions alters both preload and afterload of each lymphangion and thus mean lymph flow. To explore the effect of timing and frequency of contraction on lymph flow, we developed a computational model of a lymphatic vessel with lymphangions described by the classic description of time-varying elastance. When pumping up a pressure gradient, as in normal conditions, or when pumping down a pressure gradient, as in some cases of edema, we found that flow was optimized when the lymphangions in the vessel were pumping with a very short time delay between their cycles, and the flow was reduced when the time delay

between the contractions was reduced to zero. However, a difference in frequency between adjacent lymphangions alters instantaneous flow but does not affect mean flow. These results suggest an important role for the timing of the contraction in optimizing lymph flow. However, a difference in frequencies between adjacent lymphangions has little effect on altering lymph flow, suggesting that tight control of lymphangion coordination may not be critical for lymphatic function.

DEDICATION

This thesis is dedicated to my family who have encouraged me and provided great motivation throughout the course of this research.

ACKNOWLEDGEMENTS

I am extremely grateful to my advisor, Dr. Christopher M. Quick, for providing me with an opportunity to work under him. He gave me the option to choose a project which interested me the most. He has always given me the necessary guidance to help me complete my work, but also enough freedom to learn and produce original ideas. I am also thankful to Dr. Randolph H. Stewart, who was kind enough to let us use his experimental data for building and validating our computational model. I would also like to acknowledge the efforts of Shruti Rajagopalan in analyzing the data, which have been used to create the model. I would in addition, also like to thank the other lab members, Ketaki Desai and Mohammad Waqar Mohiuddin, who have helped during different stages of my work. Lastly, I would also like to thank my brother Arvind Venugopal who always motivated me throughout the progress of my research.

TABLE OF CONTENTS

Page

Page

LIST OF FIGURES

Page

Page

CHAPTER I

INTRODUCTION

1.1 Lymphatic vessel structure

The lymphatic system returns interstitial fluid to the blood circulation. The interstitial fluid formed by transcapillary filtration from capillaries is taken up by the initial lymphatics and is transported by the smaller lymphatic vessels to the lymph nodes. Larger conductance lymphatic vessels transport the fluid from the lymph nodes to the great veins in the neck.

Lymphatic vessels are thin-walled, contain unidirectional valves, and have muscle that allows them to contract cyclically. The unidirectional valves along the length of the lymphatic vessel ensure that lymph flows primarily from the tissues into the veins. The interstitial fluid, called lymph once it enters the lymphatic system, is transported from the lower pressure tissues to higher pressure veins during normal conditions. Since the lymph has to be transported up an axial pressure gradient (inlet pressure - outlet pressure), the valves open only when the inlet pressure is higher than the outlet pressure, thus allowing flow in only one direction.

The basic building blocks of lymphatic vessels are lymphangions, the segment of vessel between valves. The compression of the lymphatic vessel by the

---

This thesis follows the style and format of the American Journal of Physiology-Heart and Circulatory Physiology.

surrounding tissues can contribute to the increase in lymph flow by extrinsic pumping. These muscular units also cyclically contract at a frequency of 2-15 times per minute, thus providing an intrinsic mechanism to transport lymph. These cyclical contractions can thus explain the flow of lymph up a pressure gradient from the interstitial space to the venous system.

1.2 Interstitial edema

A balance is usually maintained between microvascular filtration and lymphatic drainage. Whenever the balance is affected and there is more microvascular filtration than fluid removed by the lymphatic system, there is accumulation of fluid in the interstitial space, referred to as edema. For instance, when capillary permeability increases, the pressure in the interstitial space can rise, and in extreme cases, can become higher than the pressures in veins. Similarly, venous hypertension can also lead to edema. During this condition, capillary pressure increases, leading to both increased filtration and decreased lymph flow via increased lymphatic outlet pressure. Interstitial edema is considered to be one of the primary contributors to organ failure in many cardiovascular diseases.

1.3 Lymphangion coordination

Lymphangions, often compared to ventricles (9), are affected by both preload and afterload. Increased preload can result in an increase in flow, and an increase in afterload can result in a decrease in flow (4-7). Since many lymphangions are arranged in series to form a lymphatic vessel, these small, independent pumps act

like ventricles in series, where the outlet pressure of an upstream lymphangion becomes the inlet pressure for a downstream lymphangion. The complexity of lymphangion interaction is further increased because lymphangions can have different strengths, timing and frequencies of contraction, thus affecting each lymphangion's preload and afterload. Hence, the pumping characteristics of the lymphangion are dependant on the function of the adjacent lymphangions, thus defining a significant role for coordination of contractility.

Drake et al. performed experimental studies to determine the response of lymph flow to increasing inlet (4) and outlet pressures (4-7) of entire vessels. These increases in the inlet and outlet pressures correspond to increased preload and afterload, respectively. It has been observed (Figs. 1 and 2) that, in response to an increase in preload (inlet pressure), flow increases. An increase in afterload (outlet pressure) results in a decrease in flow. However, independent manipulation of a lymphangion preload and afterload cannot be completely controlled.

There have been numerous studies (2, 10, 29) focusing on how lymphangion contractions are initiated. It is believed that spontaneous contraction of the lymphangions is initiated by stretch receptors, which are activated by the filling from an upstream lymphangion. This provides a possible intrinsic mechanism to coordinate contraction. There have also been studies showing the role of nerves (12) in modulating lymphangion contraction. However, there have been very few studies (28) focusing on how the lymphangions are coordinated and how that coordination can affect flow, since it is difficult to determine experimentally the effect of

interacting lymphangions. Investigators have observed contractions occurring in both retrograde and antegrade directions (2, 29). Uncoordinated lymphangion contraction has also been observed (2).

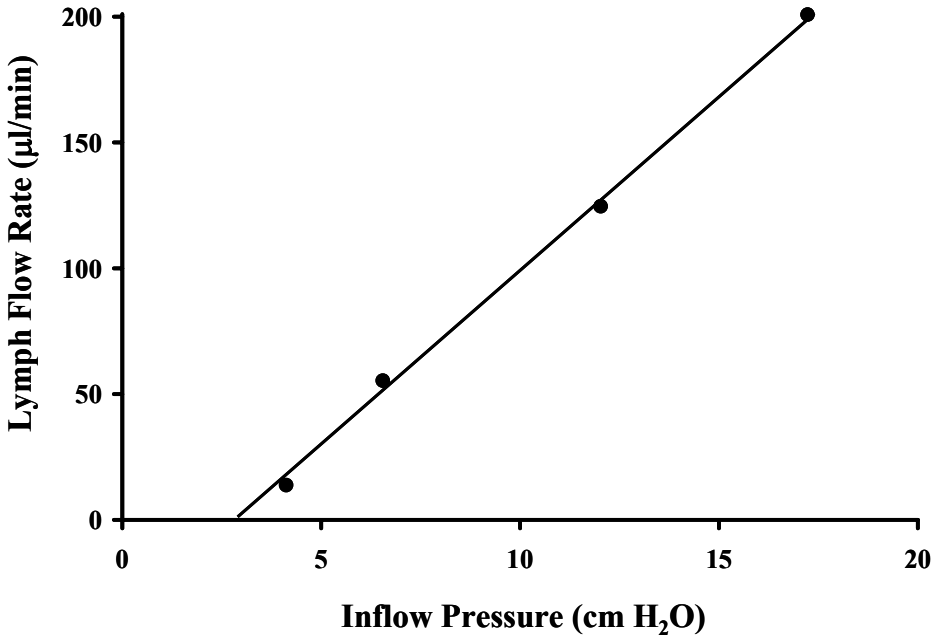

Fig. 1. Effect of increase in preload on the lymph flow. Experimental studies on dog lung lymphatic vessel showing an increase in flow with increasing preload (inlet pressure). Digitized from Drake et al., Circ Res. 50:865-9, 1982 (4).

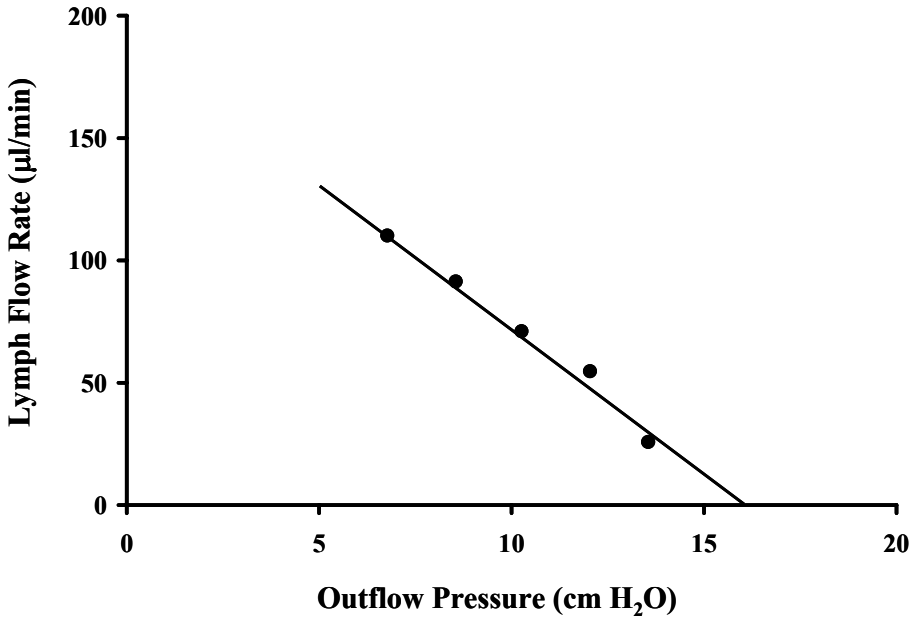

Fig. 2. Effect of increase in afterload on lymph flow. Experimental studies on dog lung lymphatic vessel showing a decrease in flow with increasing afterload (outlet pressures). Digitized from Drake et al., Circ Res. 50:865-9, 1982 (4).

These functional differences in observed coordination are mirrored by structural differences. Smooth muscle often crosses from one lymphangion to another, allowing propagation of the contraction wave through electrical coupling. Besides coordination, the particular timing of contraction may also be important indirectly through its influence on preload and afterload (3-5).

Zawieja et al. (29) performed a study to determine if the spontaneous lymphatic contraction is a propagated phenomenon, and to investigate the mechanisms by which this activity is coordinated in vessels. They observed that most of the lymphatic vessels they studied had a coordinated response with regard to contraction. The propagation of the contraction of the lymphangions was seen to

progress both from central and peripheral directions. To determine if communication exists between the adjacent lymphangions for propagation of contractions, they used a gap junction blocker (n-heptanol and oleic acid). The gap junction blocker inhibited the propagation. Thus, they concluded that gap junction communication plays an important role in the coordination of lymphangion contraction propagation.

Crowe et al. (2) studied the coordination of the contractile activity in guinea-pig mesenteric lymphatics. They also observed that the lymphangion contraction wave was propagated in both the orthograde and retrograde direction and that the contraction was highly coordinated. They reported that the coordination is due to both mechanical and electrical stimuli. A mechanical stimulus, initiated by the stretch caused by filling, stimulates stretch receptors and initiates pumping. Since filling is caused by the ejection of the adjacent lymphangions, contraction can be propagated in an antegrade direction. They also found that there was electrical activity across the valves in the lymphatic vessel, thus promoting coordinated pumping.

McHale et al. (10) performed a novel experiment on coordination of lymphangions in isolated bovine vessels. They set up a three-compartment organ bath where they could control the temperature of the individual compartments. In doing so, they controlled the direction of propagation of the lymphangion pumping. They observed that antegrade propagation and retrograde propagation resulted in similar flows. They also observed that by lowering the temperature in the middle

segment, lymphangion coordination was disrupted, indicated by different frequencies of contraction in adjacent lymphangions. However, with the above experiments they could not study how the difference in lymphangion timing affected flow.

These studies have described a role for both mechanical stretch and electrical pacemakers in lymphangion-lymphangion coordination. However the effect of the coordination on lymph flow has not been addressed.

1.4 Lymphatic vessel models

There have only been a few attempts to mathematically model lymphatic vessels. Drake et al. (5) developed a simple model to describe the lymphatic vessels consisting of an "effective resistance" and an "effective pump". They performed experiments on lymphatic vessels and determined that there is a linear pressure-flow relationship. The effective resistance is merely the slope of this relationship, and the effective pump is its intercept. This model cannot be used for describing lymphangions because it does not characterize pumping behavior. Since the model only considered the mean flow from the lymphatic vessel and not flow as a function of time, it is primarily descriptive and unable to predict how changes in radius, contractility or frequency of pumping affect flow.

A more complex approach was adopted by Reddy et al. (16, 17) who developed a distributed model. Unlike Drake's model, Reddy proposed a more fundamental model based on fluid flow equations derived from the Navier-Stokes equation.

They designed a basic model of a lymphangion, and they integrated the lymphangions to create a lymphatic system model. Therefore, from the behavior of the smallest segment in the lymphatic system, they predicted the behavior of an entire system consisting of hundreds of lymphangions. All the lymphangions in the lymphatic system were assumed to have similar structural parameters and the same timing for pumping. This model is particularly sensitive to assumptions because the slightest change in a parameter of a lymphangion can affect the whole system's pressures and flow.

Even though Reddy's lymphangion model was built using parameters which, for the most part, were based on experimental measurements, the model results were not validated with experimental data. This was particularly problematic, because lymphangion contraction was assumed, rather than characterized from experimental data. They also failed in establishing a role for coordination in their model.

A different modeling technique was adopted by Zawieja et al. who developed a more modest model to explore the effect of electrogenic and myogenic coupling of lymphangions on flow in a lymphatic vessel (28). Their model was based on time-varying elastance concept typically used to characterize ventricular contraction (19), and they assumed a representative elastance function. They had two separate models, one designed to describe electrogenic effects (compliance as a function of time) and another to describe myogenic effects (compliance as a function of wall tension) on lymph flow. They showed that the electrogenic model was sensitive to preload. They predicted an increase in stroke volume with increase in preload. However there was

no increase in stroke volume observed in the myogenic model. The frequencies, however, varied with an increase in preload. They showed that both electrogenic and myogenic models could capture some of the behaviors of lymphangions while the other one could not. Hence they concluded that the lymphangions may actually have both the electrogenic and myogenic characteristics. Even though the model describes the lymphangion contractions, they could not validate the model flows due to the inability to measure flow from the microlymphatics.

The mechanisms of coordination have been studied extensively, but the effect of coordination on flow has not been addressed due to the inability to control the necessary variables in an experimental setup. Some mathematical models have also been developed for better understanding the function of lymphangions and lymphatic vessels (5, 16-18). However, the few studies to determine the influence of coordination on flow have been incomplete.

1.5 Similarity to veins, arteries and ventricles

Lymphangions have been shown to have the characteristics of both blood vessels and the heart. For instance, there is a very striking structural symmetry with veins. They are thin walled like the veins and can collapse under negative transmural pressures. They also have unidirectional valves, aiding the transportation of lymph from the interstitial space to the venous system.

Lymphangion function has also been studied using ventricular analogies. Lymphangions, which contract to propel lymph, have been shown to have pressure-volume loops similar to that of the heart (9). Lymphangion pumping has been

characterized by systolic and diastolic phases, stroke volume and ejection fraction (1, 9).

Successful mathematical models of the heart and the blood vessel provide a wealth of concepts available to construct a lymphangion model. Ventricular pressure-volume loops, in particular, have been used by investigators to construct mathematical models of cyclically contracting chambers.

1.6 Ventricular pressure-volume loops

Characterization of ventricular pressure-volume loops has been proposed as a means to measure the ventricle's contractile properties. Frank showed the first pressure-volume loops from a frog ventricle. Fig. 3 shows a schematic of a ventricular pressure-volume relation of a ventricle for one cardiac cycle. To understand how a pressure-volume loop can describe the function of the heart, a simple description is given below.

There are four different regions associated with the curve. Starting from A, corresponding to end-diastole, the ventricle begins to undergo isovolumic contraction to point B, where the aortic valve opens due the pressure gradient across the valve. The ventricle then goes through the phase of ejection which corresponds to the B-C region. Then the ventricle progresses through an isovolumic relaxation phase to point D, where the pressure in the ventricles falls below the atrial pressure. The mitral valve opens, and ventricle starts to fill in the D-A portion of the curve. The region between D and A in the volume axis represents the stroke volume of the

heart, and the area inside the pressure-volume loop is the stroke work done by the heart.

1.7 Influence of preload and afterload on cardiac function

Preload and afterload influences the performance of the heart. Preload is the hemodynamic load on the myocardial wall at the end of diastole just before the contraction begins. Afterload is the hydraulic load imposed on the ventricle during ejection. This is typically the load which the heart has to pump into the arterial system. Any changes in systemic vascular impedance will affect the afterload.

1.8 Time-varying elastance to characterize contractility

Suga et al. (22) proposed time-varying elastance as a descriptive model to characterize the functioning of the heart independent of preload and afterload. They described the ventricular elastance, E(t), as the ratio between the instantaneous pressure, P, and the volume, V,

$$E(t) = \frac{P(t)}{V(t)} \quad , \tag{1}$$

They obtained left ventricular P-V loops under various end-diastolic volumes and aortic pressures in anaesthetized, thoracotomized dogs. Left ventricular instantaneous volume was calculated from the aortic flow and ejection fraction at a particular cardiac contractile state.

The elastance obtained is based on the P-V loops at different preloads, yielding an end-diastolic pressure volume relationship (EDPVR) and end-systolic pressure volume relationship (ESPVR). The ESPVR can be represented as a straight line.

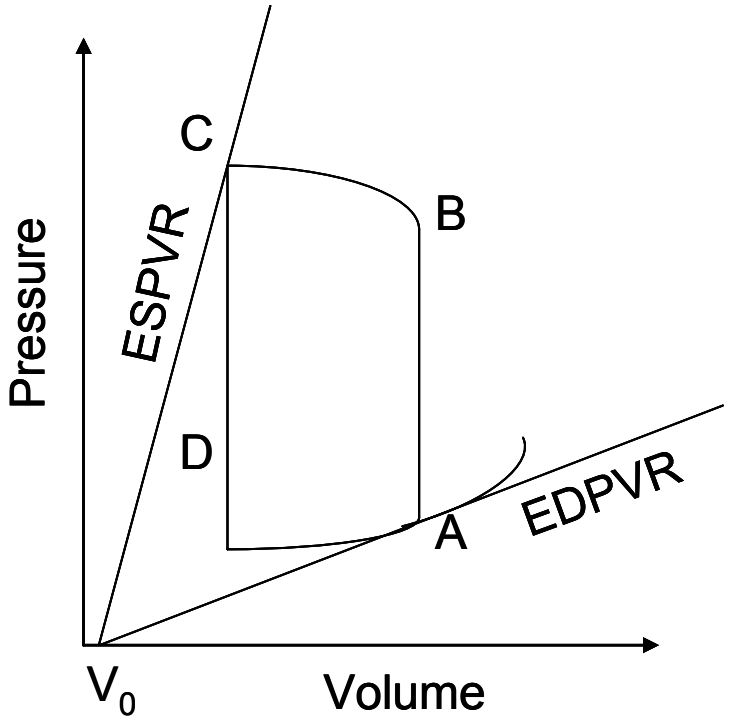

Fig. 3. A representative pressure-volume loop of the ventricle. Point A represents the end of diastolic phase, point C represents the end of systolic phase.

The slope of the line is equivalent to E_{max}, the maximum value of E(t). An increase in E_{max} is observed with positive inotropic stimulation, and therefore represents an index of contractility. The EDPVR represented the pressure-volume relation of the

heart in the most relaxed phase. However, the EDPVR is not as linear as the ESPVR and tends to be exponential. Figure 3 shows the EDPVR and ESPVR. The model assumes a linear ESPVR as well as a linear EDPVR. Both the EDPVR and ESPVR have a minimum volume below which the heart fails to create any pressure. Even though these volumes are not the same, little error is incurred by assuming that they are equal. The term that represents the volume at zero pressure is called the dead volume, V_0, and is added to the original elastance equation as a correction factor (21). Thus, the preferred equation for ventricular elastance is

$$E(t) = \frac{P(t)}{V(t) - V_0}. \tag{2}$$

There are numerous advantages associated with the use of the time-varying elastance model. It describes the maximum force of contraction, E_{max}, and the model can predict both the pressures that the heart can develop and flows that the heart pumps in response to changes of both preload and afterload. Hence, time-varying elastance concept is a popular foundation for cardiovascular models.

Even though there are numerous advantages associated with this model to describe the heart, there are some problems with its use. Since this elastance model is a complete representation of the heart as a whole, the model will fail under conditions like ischemia where the mechanical characteristics of the ventricles are different in the ischemic region than other parts of the ventricle. To describe regional ischemia, multi-chambered models were introduced (23).

The elastance defines both the pressures and flows from the ventricle. Suga et al. suggested a model (21) in which the pressure is defined as a function of volume. This elastance can successfully describe the Frank-Starling mechanism observed with the ventricles. However, the pressure generated by the ventricle is diminished by rapid changes in volume, due to the Hill effect, making ventricular pressure also sensitive to aortic flow (14). Shroff et al. (20) showed that an additional "internal resistance" improved the performance of Suga's basic time-varying elastance description.

1.9 Models of blood flow in arteries

Modeling of the arterial system has been of great interest over the years. The simplest arterial system model consists of a resistor, representing the peripheral resistance. This model is useful in relating the mean values of pressures and flow. To better the peripheral resistance description of the arterial system, Frank described it as a Windkessel. According to this description, large arteries were modeled as a single compliant chamber. He assumed that the vessels are compliant and hence can store blood volume. He based the description on the concept of conservation of mass,

$$Q_{in} = C\frac{dP}{dt} + \frac{p}{R}, \tag{3}$$

where Q_{in} represents the flow into the arteries, C is the compliance of the chamber with a pressure P, and R is the resistance that the arterial segment offers to flow.

The Windkessel model proved to be a good description of the arterial segments for low frequencies. However, the model could not describe the high-

frequency components of pressure and flow. Hence, there was another model developed in its most complete form by Womersley (27). He described the arterial segment as an infinitely long tube. Even though this model is a better description in high frequencies, it gives a poor description in low frequencies.

There was another approach suggested by Noordergraaf in describing flow in a vessel segment. The equations used were derived from simplifying Navier-Stokes equations. He described the arterial segment using the fundamental principle of conservation of momentum. He used a resistor to describe the resistance (R) that the vessel offers, which was given by Poiseuille's Law and an inertance (L) to describe the inertial effects due to the momentum. An electrical analogy of the model is as shown in Fig. 4. The resulting equation of motion is

$$L\frac{dQ}{dt} + RQ = P_{in} - P_{out}\,, \tag{4}$$

and equation of continuity is

$$Q = C\frac{dP_{out}}{dt}\,. \tag{5}$$

C describes the compliance of the vessel. The resulting approximations of pressure-flow relationships allow investigators to avoid computational fluid dynamics, yet provides a description that captures most of the characteristics of the blood vessel (13).

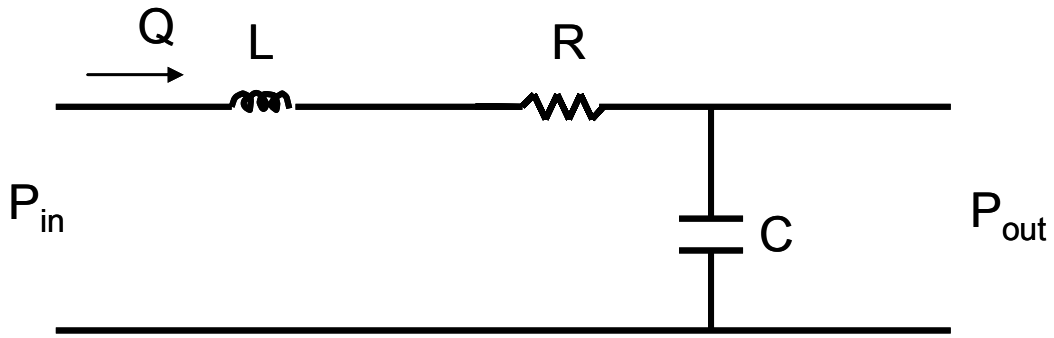

Fig. 4. Transmission line description of the blood vessel. R, L and C represents the vessel resistance, inertance and the compliance.

CHAPTER II

PROBLEM STATEMENT

The lymphatic system is made up of many individual lymphangions that can potentially act independently. The coordination between the lymphangions may be a critical factor in lymphatic system function and a target for treating edema. Similarly the dis-coordination between the lymphangions, i.e. the case where the inherent frequency of pumping between the adjacent lymphangions are different, may be a pathological condition. We cannot experimentally determine the role of lymphangion coordination in determining lymph flow from the tissues.

The goal of this project was to determine the effect of coordination of lymphangion contraction on lymph flow. To do so, a mathematical approach was taken to construct a lymphangion model, based on actual measured properties of lymphangions and fundamental physics of fluid flow, which was then used to construct a lymphatic vessel model. By evaluating the effects of difference in timing and frequency between the lymphangions in the vessel, we have determined the relevance of coordination on lymph flow. Although this study is largely model-based, experimental data has been used in both building and validating the model.

As mentioned in Chapter I, there have been very few studies focusing on how coordination affects flow, due to the inability to manipulate the timing and frequency of lymphangion contraction. Hence, in order to determine the effect of coordination

on lymph flow, a mathematical model that relates structural properties of lymphangions to lymph pressure and flow is needed.

In Chapter III, we develop the lymphangion model. This model is based on the concept of time-varying elastance typically used to describe ventricles. The elastance will be based on data obtained from experiments conducted in bovine mesenteric lymphatic vessels. This model will give us the power to change the values of parameters that cannot be altered experimentally.

Chapter IV will discuss how well the model predictions match the experimental results. The validation of the model will justify the techniques used in the development of the model. The specific cases for which the model flows will be validated are

1. Lymphangion model subjected to boundary conditions of positive and negative axial pressure gradients.
2. Lymphatic vessel with different states of coordination in lymphangion contraction.

This validated model will be used to determine how the time delays and frequency of contractions between adjacent lymphangions in a vessel affect flow.

CHAPTER III

MODEL DEVELOPMENT

3.1 Time-varying elastance for lymphangions

Due to the similarities of the function of lymphangions to the heart, investigators (1, 9) have described lymphatic function using parameters such as stroke volume and ejection fraction. Li. et al (9) also showed the pressure-volume loops in a lymphangion. Even though lymphangions function like ventricles, they are structured like blood vessels. Lymphangions have a cylindrical structure and the flow through them is dependant on the pressure gradient. Thus, lymphangions can also be described as a vessel. We have developed a model where we describe the lymphangions both as a ventricle, with a time-varying elastance, and as a vessel, where the flow is governed by the pressure gradient, time-varying resistance, inertance and compliance. The time-varying elastance model was shown to be a model independent of both preload and afterload (22). Lymphangion's functional parameters also depend on both preload and afterload. The ESPVR is linear similar to that of the heart and the EDPVR is more exponential than the heart.

Hence, in order to characterize the lymphangions which have pressure-volume loops similar to the heart, the time-varying elastance concept (Eq. 6) can be used to describe their functional behavior. However, the time-varying elastance description, which can adequately describe ventricular function, is yet to be established in the case of lymphangions.

Time-varying elastance is defined as the time-varying ratio of Pressure (P) and Volume (V),

$$E(t) = \frac{P(t)}{V(t) - V_0}, \tag{6}$$

where V_o is the dead volume.

The time-varying elastance is fundamentally a descriptive model, merely characterizing the pressure-volume relationship. However, its application to lymphangions requires a number of implicit assumptions. The time-varying elastance concept provides a description of maximum contractility, E_{max}, and this is given by the end-systolic pressure-volume relationship (ESPVR). The ESPVR can be adequately approximated as a linear function. However, the lymphangion end-diastolic pressure volume relationship (EDPVR) has a more pronounced exponential relationship than that of the heart, which deviates from a linear relationship at higher pressures.

This model is based on the elastance calculated from the pressure and diameter data obtained from *in vitro* experiments on bovine mesenteric lymphatic vessels. The data were obtained from experiments in which post-nodal mesenteric lymphatic vessels were isolated from freshly slaughtered cattle and placed in a tubular organ bath. The vessels were perfused with a balanced polyionic solution gassed with 95% O_2-5% CO_2 sufficient to maintain pH at 7.4. The length of the vessel segment (l) will be the length of the organ bath and was set at 4.2 cm. The inlet and outlet pressures (P_{in}, P_{out}) were set and diameter (2r) of the vessel was

measured continuously using a video dimension analyzer. Flow was measured using a flowmeter near the outlet end as described in Fig. 5.

The video dimension analyzer was developed in LabVIEW. We used a camera to capture the video of the lymphatic vessel. The image is fed into the computer through an image acquisition board. The image was obtained by LabVIEW software and, with the help of an edge detection algorithm, real time changes in diameter were estimated and recorded. The distance between the edges was calculated in pixels. That distance was calibrated and converted to centimeters.

Volume (V) was calculated from the measured diameter assuming that the lymphangion maintains a uniform cylindrical shape during contraction.

$$V = \pi \cdot r^2 \cdot l \ . \tag{7}$$

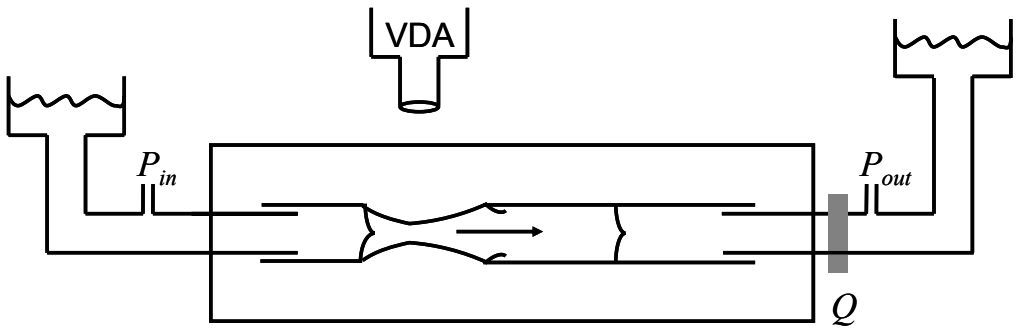

Fig. 5. Experimental setup used to measure the diameter via video dimension analyzer (VDA), the pressures (P_{in}, P_{out}) and flow (Q).

3.2 Experimental protocol

The experiment was conducted on a lymphatic vessel with only one valve, which allowed us to measure the chamber pressure by measuring the pressure at the outlet end (neglecting the small vessel resistance). Input pressure was set at 5 mmHg and the output pressure was varied to obtain axial pressure gradients between 1 and 3 mmHg. The pressure ranges were chosen to be approximately 5 mmHg for two reasons. First, it represents the higher pressures found in edema, yet it is below reported levels inducing pump failure (11). The diameter of the lymphangion was measured using the video dimension analyzer. Volume was calculated as in Eq. 7.

3.3 Data analysis

The measured pressure and the calculated volume were used in the calculation of elastance (Eq. 6). The obtained elastance was analyzed and a representative elastance curve was obtained from the family of curves. Pressure-volume loops were obtained and the end-diastolic pressure-volume relationship was found to be exponential. The ESPVR was obtained by fitting a line to the end-systolic points in the loop. The dead volume, the intercept of the ESPVR and the volume axis (representing the theoretical volume below which there is zero pressure), obtained from the end-systolic pressure-volume relationship (Fig. 6) was found to be 0.05 cm^3. The pressure and volume data used in the calculation of the representative elastance are shown in Figs. 7 and Fig. 8, respectively. The last two data points in the calculated elastance (Fig. 9) were manipulated to make the starting and the ending data points match, ensuring stability during simulations.

3.4 Lymphodynamics

The lymphangion model is based on the characteristics of both the ventricles and blood vessels. Lymphangion pressure and volume data obtained are used as the core of the model, and the derived time-varying elastance will provide the contractile characteristics to the lymphangion model. The resulting model is similar to the classic transmission line description of arteries (13), but with time-varying compliance (Fig. 10). Furthermore, the time-varying resistances and inertances are convenient descriptions of the resistance that the vessel offers to flow and the inertial effects of the lymph.

The difference in the inlet pressure and the pressure at the center, marked as P (Fig. 10), defines the flow in the inlet section (Q_{in}) of the lymphangion. This is given by

$$P_{in} - P = E(t) \cdot (V - V_0) + R_{in} \cdot Q_{in} + \frac{R}{2} \cdot Q_{in} + \frac{L}{2} \frac{dQ_{in}}{dt}, \quad (8)$$

where *E(t)* represents the elastance given by Eq. 6. The flows in the system are calculated by differentiating the volume (*V*), assuming conservation of mass,

$$\frac{dV}{dt} = Q_{in} - Q_{out}, \quad (9)$$

where R_{in} represents the inlet resistance of the organ bath and was estimated to be 143.5 dyne·s·cm^{-5}.

R is the resistance of the lymphatic vessel which is calculated using Poiseuille's law,

$$R = \frac{8l^3\pi\mu}{V^2}, \tag{10}$$

where μ is the viscosity of the Krebs solution used, and l is the length.

Inertance, L, is calculated using the density of the fluid (ρ) flowing through it and is given by

$$L = \frac{\rho \cdot l^2}{V}. \tag{11}$$

The resistance and the inertance which are given by these equations are functions of volume. Unlike the description of resistance and inertance in standard transmission line theory, a decrease in volume due to the contraction can result in higher resistance and inertance, both of which are treated as functions of time.

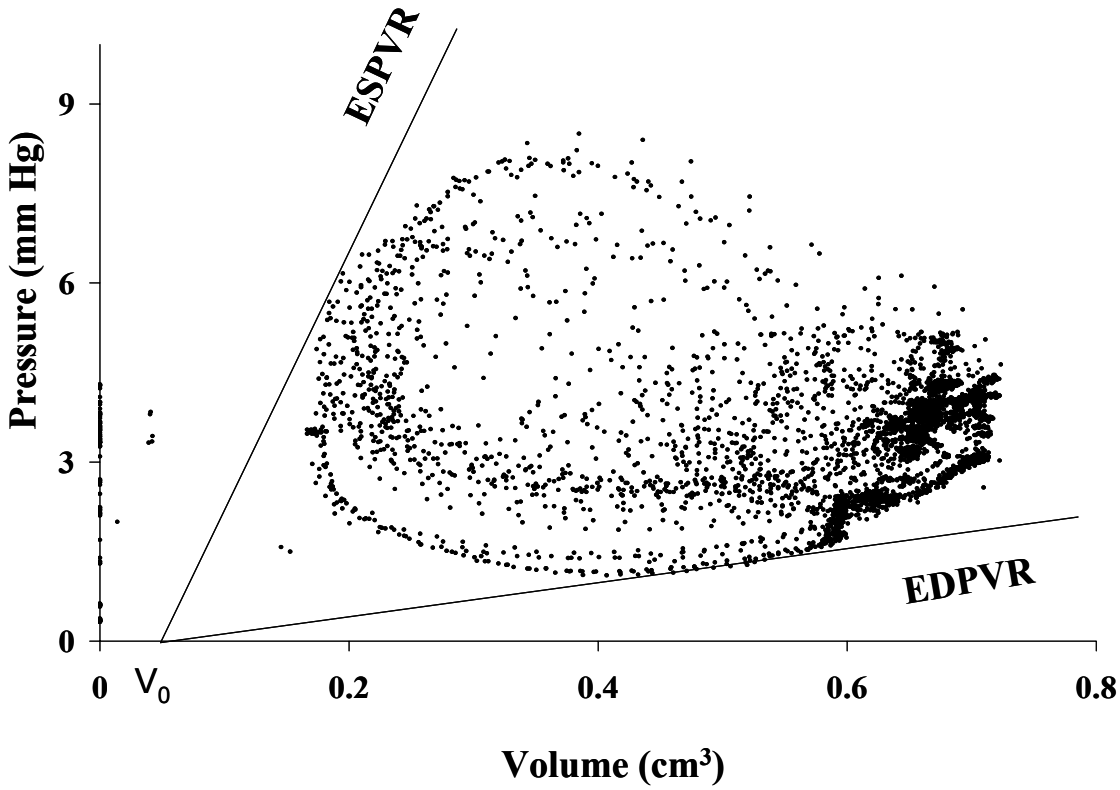

Fig. 6. Pressure- volume loops obtained from the lymphatic vessel segment with one valve.

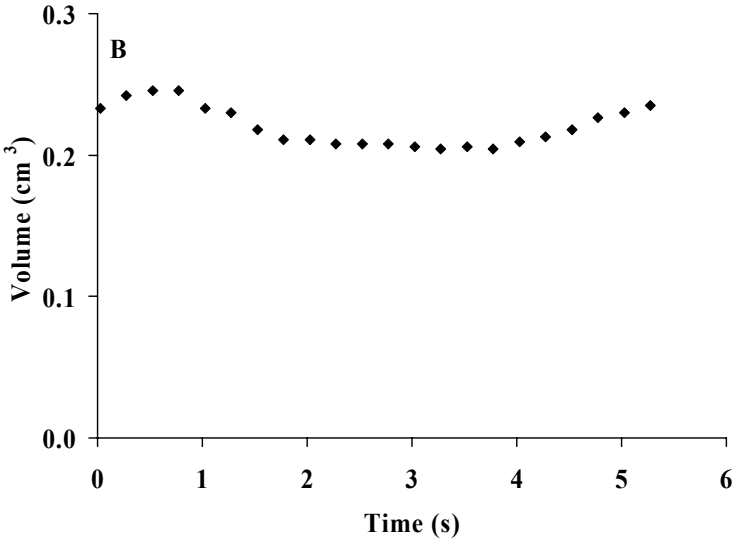

Fig. 7. Volume as a function of time. Volume calculated from the measured diameter of the bovine mesenteric lymphatic vessel. Venugopal et al.(26) (© 2003 IEEE)

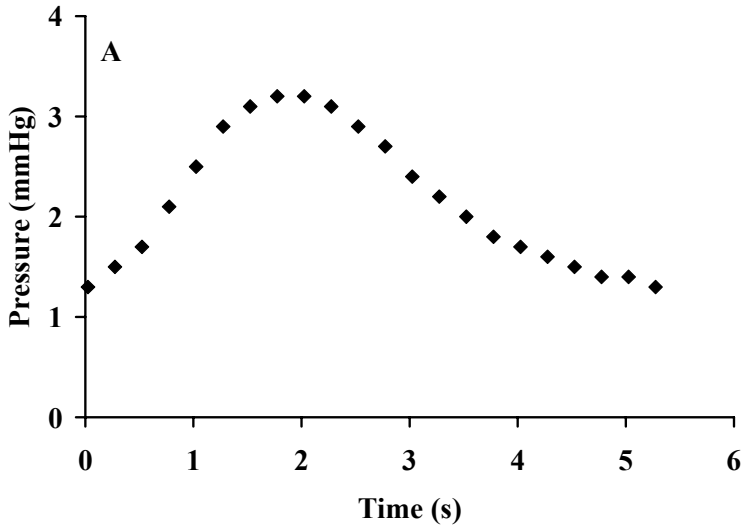

Fig. 8. Transmural pressure of bovine mesenteric lymphatic vessel. Venugopal et al.(26) (© 2003 IEEE)

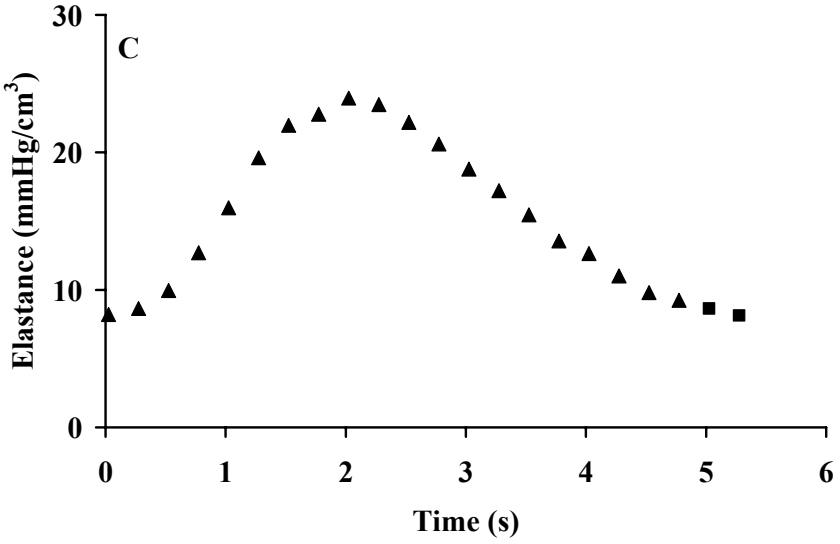

Fig. 9. Time-varying elastance calculated using pressure and volume. Two data points (■) added to ensure that beginning and end data points match. Venugopal et al.(26) (© 2003 IEEE)

The difference in pressure between P and the outlet pressure will determine the flow out (Q_{out}) of the lymphangion,

$$P - P_{out} = E \cdot (V - V_0) - R_{out} \cdot Q_{out} - \frac{R}{2} \cdot Q_{out} - \frac{L}{2} \frac{dQ_{out}}{dt}, \quad (12)$$

where R_{out} represents the outlet resistance of the bath and was measured to be 1218.2 dyne·s·cm^{-5}.

When we equate the pressures at the inlet and outlet end, the resulting equation can be written as

$$\begin{aligned} &P_{in} - P_{out} = \\ &R_{in} \cdot Q_{in} + \frac{R}{2} \cdot Q_{in} + \frac{L}{2} \frac{dQ_{in}}{dt} \\ &+ R_{out} \cdot Q_{out} + \frac{R}{2} \cdot Q_{out} + \frac{L}{2} \frac{dQ_{out}}{dt} \end{aligned} . \quad (13)$$

The above equation, along with description of unidirectional valves, describes the functioning of the lymphangion. The unidirectional valve was represented by a diode with a forward resistance and inertance. The resistance value used in the model was assumed to be 10 dyne·s·cm^{-5} and an inertance of 0.05g/cm^4. The flow from the lymphangion model is sensitive to assumed parameters, which are listed in Table1.

The above equations used for the lymphangion, have been described using equations that were developed for both blood vessels and ventricles. The transmission line description of the blood vessel, with its approximations of the pulsatile pressure-flow relationship, allows us to describe a small segment of vessel with a rather simple set of equations.

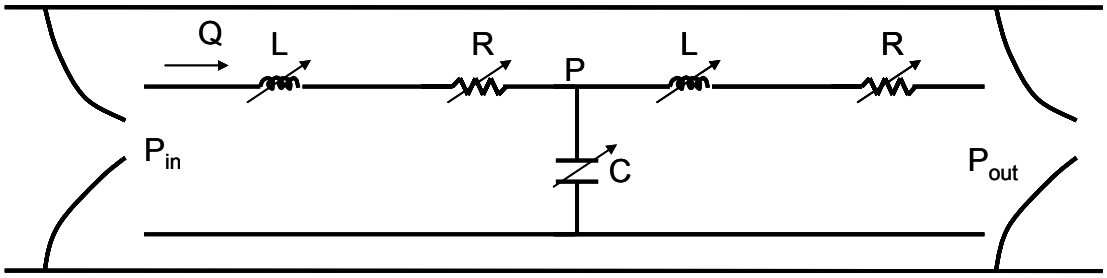

Fig. 10. Lymphangion model characterized by a time-varying resistance, R (t), inertance, L (t) and elastance, E (t).

All the equations were solved in Simulink, a graphical programming tool that can solve equations describing dynamic systems. This software allows us to couple

lymphangion models to obtain a model of an entire lymphatic vessel. This feature is absent in most of the other software where the equations have to be rewritten in order to build complex models from simpler components. Furthermore, Simulink allows us to use electrical components using Simpower system, in addition to the equations we explicitly enter, allowing electrical equivalents to be used to represent the valves and the pressure source.

As illustrated in Fig. 10, the lymphangion can be represented in terms of its electrical equivalent circuit where the pressures and flows are analogous to the voltage and currents, respectively. To describe a unidirectional valve, the electrical equivalent, the diode was used. The function of the diode is to let the current flow through them when there is a positive pressure gradient (inlet pressure > outlet pressure). Hence, this diode represents the one way valves allowing flow only when there is a positive axial pressure gradient across the valve.

Table 1. List of the important parameters used in the model and its values.

Parameters	Parameter values
Inlet Resistance (R_{in})	143.5 Dynes.s/cm^5
Outlet resistance (R_{out})	1218.2Dynes.s/cm^5
Maximum elastance (E_{max})	23.95 mmHg/cm^3
Minimum Elastance (E_{min})	8.12 mmHg/cm^3
Length of the vessel (l)	4.2 cm
Dead Volume (V_0)	0.05 cm^3
Frequency (f)	678 s^{-1}
Time delay (Δt, Lymphatic vessel)	0-5.275 s

CHAPTER IV

MODEL VALIDATION

Reddy et al. developed a large-scale lymphatic system model from individual lymphangions, but failed to validate its use in the whole system. Furthermore, their predictions of the complex system were based on the simple description of the individual lymphangions, which were themselves invalidated. To avoid these difficulties, both the lymphangion and the lymphatic vessel models were validated to justify their use in revealing the role for coordination on lymph flow.

To test if the lymphangion model predicts observable phenomena, we performed modeling experiments analogous to the actual *in vitro* experiments. Similar boundary conditions were used, allowing us to compare the results from the model with that of the experiments. To validate the flows from the lymphatic vessel model when lymphangions were dis-coordinated, a particular experimental case where a state of dis–coordination was gleaned from previously recorded data and was compared with the model with similar contraction frequencies.

4.1 Lymphangion model validation

4.1.1 Experimental setup

The experiments to which the modeling results are compared were performed on a bovine mesenteric lymphatic vessel. The *in vitro* setup is the same setup used to characterize elastance (Section 3.1). However, these experiments were performed in lymphatic vessels with more than one valve, allowing them to generate a flow.

4.1.2 Modeling setup

The lymphangion model boundary conditions are set very similar to the *in vitro* setup. The lymphangion model was given a resistance in the inlet and outlet resistance similar to that of the vessel bath. The inlet pressures and the outlet pressures were set, thus defining an axial pressure gradient. Flow is governed by variables like resistance, inertance and compliance given by the equations defined above.

4.2 Experimental protocol

4.2.1 Flows during positive and negative axial pressure gradients

To determine the flow from the lymphangion during negative axial pressure gradients, the inlet pressure was set at 5 mmHg, and the outlet pressure at a higher value of 6 mmHg. The instantaneous flows were measured using a flowmeter and is shown in Fig. 11. This is representative of the pressure gradient that the lymphangions are subjected to during edematous conditions, where the tissue pressure is lower than the venous pressure. (see Section 1.2.)

To compare the result of the experiment with that of the model, the lymphangion model was set to a similar pressure gradient. The model prediction of flow during negative ($P_{in}<P_{out}$) axial pressure gradient was compared by calculating flow as a function of time as shown in Fig. 12.

To obtain flow during positive axial pressure gradients, the lymphangion in the *in vitro* preparation is subjected to an axial pressure gradient where the inlet

pressure is higher than the outlet pressure. Hence the inlet pressure is set at 5 mmHg and outlet pressure at 4 mmHg. The measured flow was obtained as a function of time and is given as in Fig. 13.

The model prediction of flow during positive ($P_{in}>P_{out}$) axial pressure gradient was compared by calculating flow as a function of time as shown in Fig. 14.

Both the experimental results and the model predictions suggest that when the inlet pressure is greater than the outlet pressure, there is flow from the lymphangion only during systole. Hence, if the lymphangion fails to cyclically contract, there will be no flow. However, with a negative axial pressure gradient, when the outlet pressure is greater than the inlet pressure, there is flow from the lymphangion, even during diastole. This suggests that lymphangion contraction is not necessary for the lymph to flow when the lymph is flowing down a pressure gradient.

4.2.2 Effect of axial pressure gradient on flow

Experiments were conducted in the *in vitro* set up to determine the effect of axial pressure gradient on flow. The input pressure was set at 5 mm Hg, and the outlet pressure was varied such that the pressure ranges covered positive and negative axial pressure gradient. The arithmetic mean of the flow thus calculated was obtained and plotted as a function of the pressure gradient as shown in Fig. 15. The lymphangion was then perfused with a calcium-free solution to make the lymphangion passive. After the lymphangions stopped cyclically contracting, the protocol was repeated.

An analogous protocol was also carried out with the mathematical model. Instantaneous flows were averaged and were plotted as a function of the axial pressure gradient as in Fig. 16. Then, to make the lymphangion behave like a passive vessel, the elastance was set to a constant equal to the minimum elastance, assuming that passivation of the lymphangion only effects E_{max}. The protocol was repeated for the lymphangion model with passive characteristics.

The above figures show the rapid increase in flow when the lymphangion switches from a negative axial pressure gradient to a positive one. This "switch" can be attributed to the lymphangion's behavior having the characteristics of both the pump and the vessel. As mentioned before, the lymphangion has to pump in order to transport lymph when they are subjected to negative axial pressure gradient. However, as we can observe from Figs. 15 and 16, lymphangion pumping can actually inhibit flow when subjected to positive axial pressure gradients.

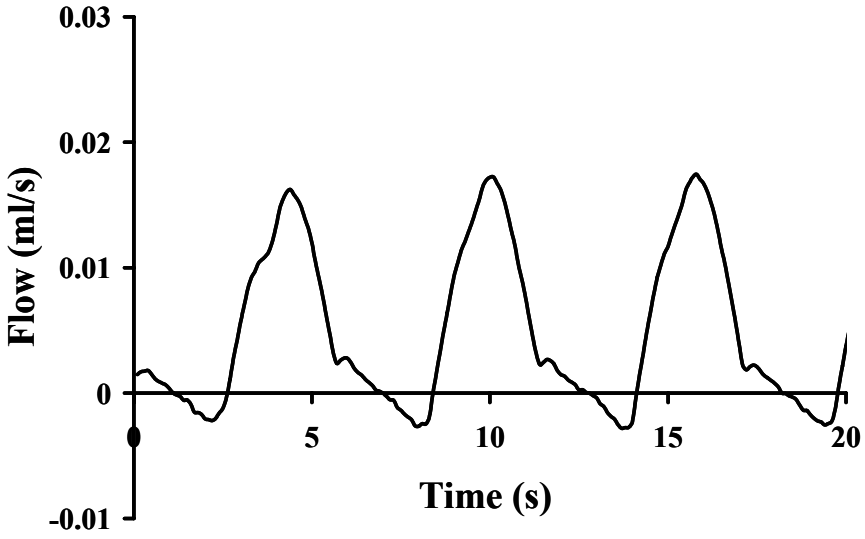

Fig. 11. Flow from isolated bovine lymphatic vessel subjected to negative axial pressure gradient. Negative flows are due to the segment of lymphangion after the valve near the outlet end allowing flow backwards during diastole.

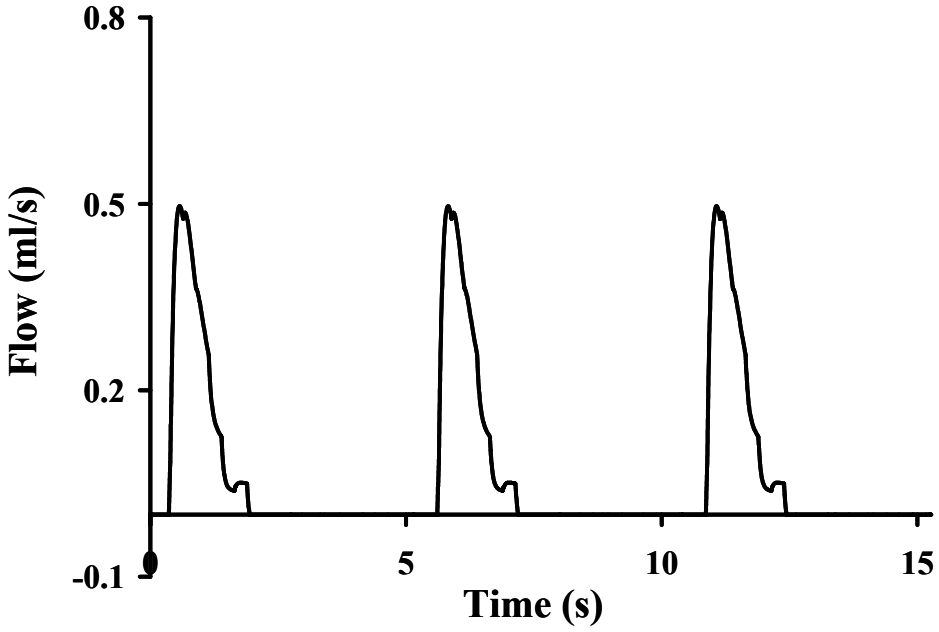

Fig. 12. Flow from the lymphangion model subjected to negative axial pressure gradient.

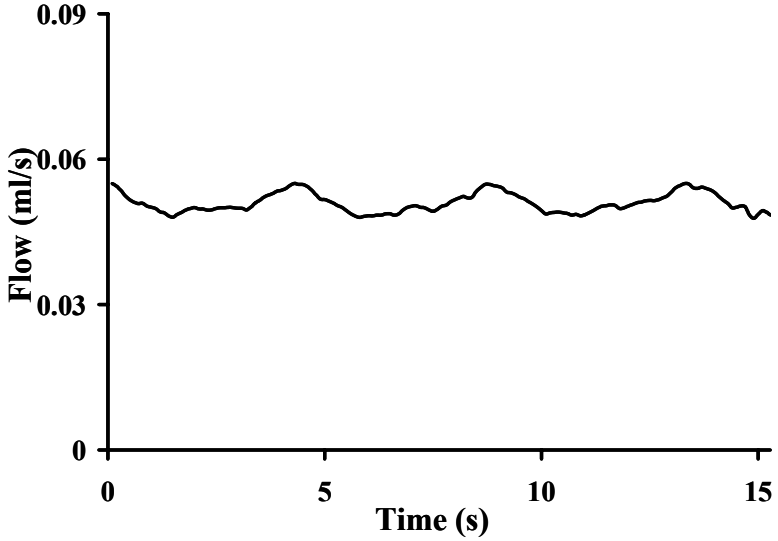

Fig. 13. Flow from isolated bovine mesenteric lymphatic vessel subjected to positive axial pressure gradient.

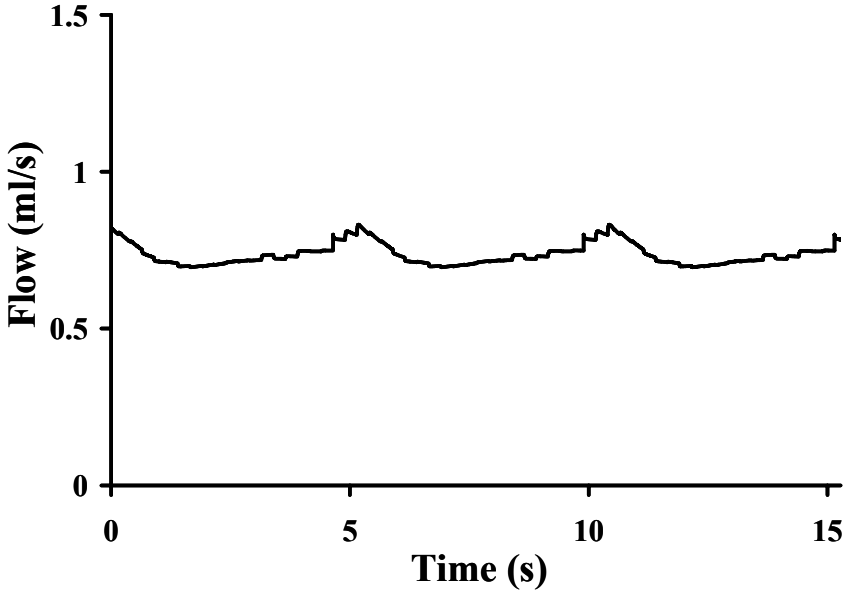

Fig. 14. Flow from the lymphangion model subjected to positive axial pressure gradient.

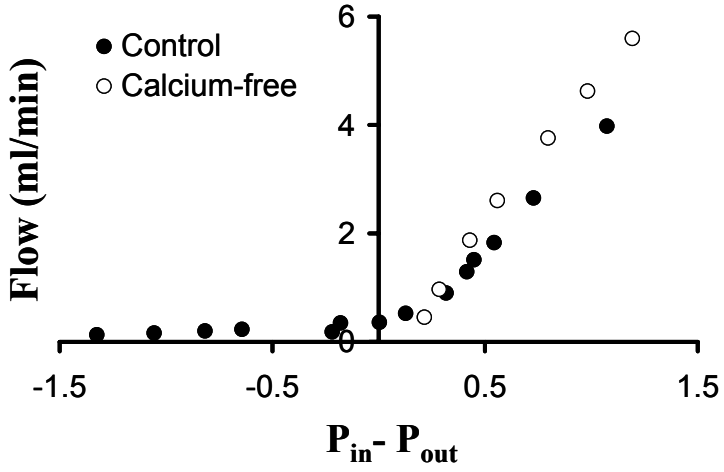

Fig. 15. Flow measured in actively contracting lymphangion when subjected to positive and negative axial pressure gradients.

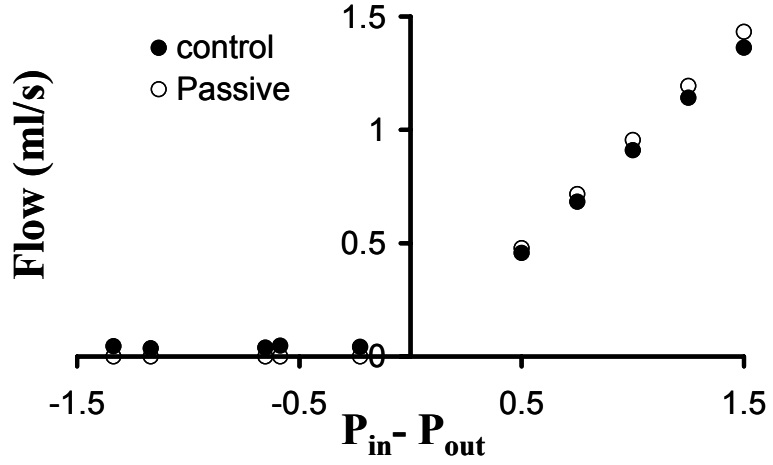

Fig. 16. Calculated flow from the lymphangion model when subjected to positive and negative axial pressure gradients.

4.3 Validation of the lymphatic vessel model

The experiments are performed on lymphatic vessels that have lymphangions contracting with similar frequencies, and the frequency of an individual lymphangion cannot be altered easily. Hence, to validate the lymphatic vessel model for different states of coordination, experimental data are limited to vessels that exhibit a difference in frequency between the adjacent lymphangions.

4.3.1 Experimental protocol

The same *in vitro* experimental setup was used to obtain flows from a lymphatic vessel with two lymphangions. The frequencies of the pumps were observed to be different. The ratio of their frequencies (F_1 /F_2) was calculated to be 0.92. The lymphatic vessel was made to pump up a negative axial pressure gradient with outlet pressure greater than the inlet pressure. The measured flow from the lymphatic vessel is illustrated in Fig. 17. We observe negative flows because the flow meter is on the outlet end, and there is a small segment of a lymphangion on the outflow side between the last valve and the flowmeter, allowing a reversal of flow when it relaxes.

4.3.2 Modeling protocol

A lymphatic vessel model consisting of two lymphangions was validated by matching it to the experiment. The frequencies of the lymphangions were set such that the ratio of their frequencies was the same as that calculated from the experiment (F_1/F_2 =0.92). The flows were calculated given pressure gradients similar

to those of the experiments. The resulting flow from the lymphatic vessel model is as given in Fig. 18.

The lymphatic vessel model shows similar flow patterns to the experimental data, thus validating the model. The model results for flow exhibits the same beat frequency as exhibited by the experiments. Since the ratio of the frequencies were matched and not the actual frequency calculated from the experimental data, only the flow pattern matches and not the actual time course of the flow.

This lymphatic vessel model is used to evaluate the effects of the difference in frequency on flow in addition to estimating flow as a function of time delay. These two parameters, frequency difference and time delay, will allow us to determine the effects of coordination on lymph flow.

The effect of frequency difference between the lymphangions has a time-dependent effect on flow. Model predictions of the behavior of the flow match the experimental observation. However, the amplitudes of the flow obtained from the model have much higher values.

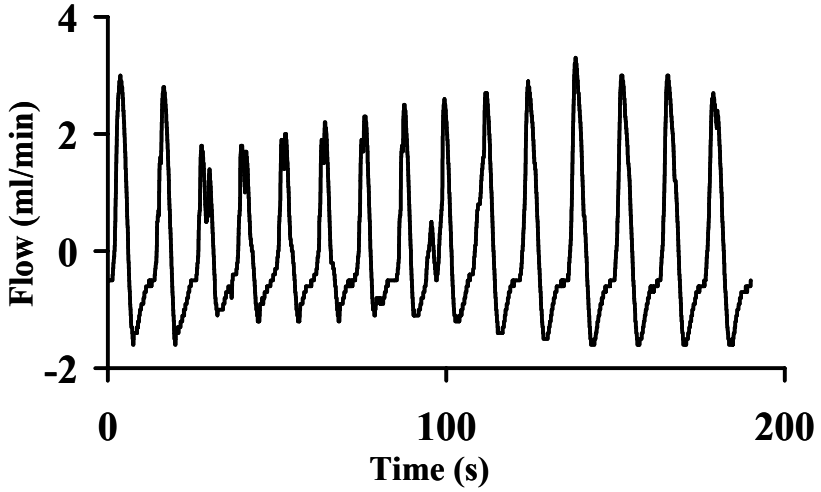

Fig. 17. Flow from a bovine lymphatic vessel having lymphangions with different frequencies. The flow from the lymphangion was measured with the input pressure set to 5 mmHg and the output pressure set to 6 mmHg(24).

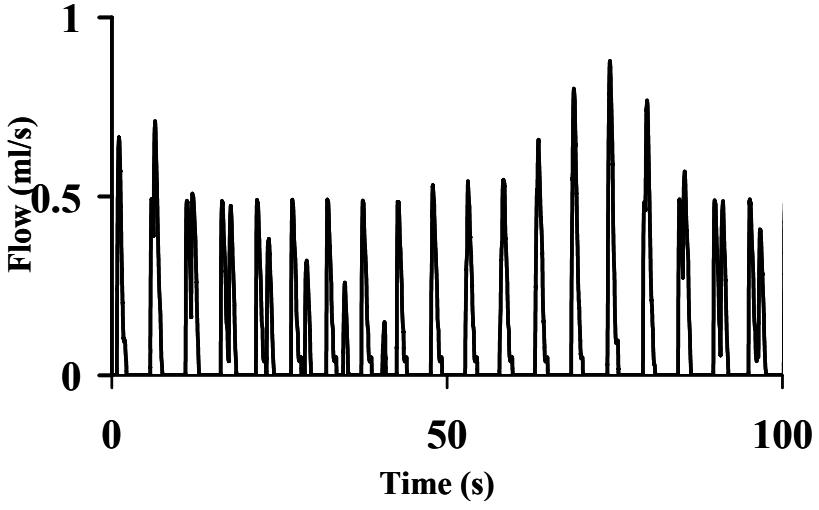

Fig. 18. Lymphatic vessel model validation. The flow from the lymphangion was simulated with the input pressure set to 5 mmHg and the output pressure set to 6 mmHg(24).

CHAPTER V

EFFECTS OF COORDINATION ON LYMPH FLOW

The validated lymphatic vessel model was used in determining the effects of contraction timing and frequency on lymph flow. Lymphangion flow is affected by changes in preload and afterload. The difference in the timing of contraction can bring about changes in preload and afterload, and so can the difference in frequency. The structural parameters like timing and frequency of contraction cannot be altered experimentally. Hence, to determine the effect of timing and frequency on lymph flow the lymphatic vessel model provides a particularly useful tool.

5.1 Effect of timing on lymph flow

5.1.1 Modeling methods

To determine the effect of timing on lymph flow, multiple lymphangions were connected in series to model a lymphatic vessel (Fig. 19). The simulation was performed for six lymphangions in series, because the behavior of the vessel did not change appreciably by adding more.

All the lymphangions were made to pump with a set time delay (Δt) between the start of contraction of one lymphangion to the start of contraction of the following lymphangion. The time delay is incorporated in the model by adjusting the time curve of the elastance, which defines the contraction of the lymphangion in the model.

$$E_1(t) = E_2(t + \Delta t) = E_3(t + 2\Delta t) = E_4(t + 3\Delta t)\ldots \quad (14)$$

This set time delay between the lymphangions makes the lymphangion pump one after the other, either in the antegrade direction or the retrograde direction.

The pressure at the input end was set at 5 mmHg. The outlet pressures were set at either 6 mm Hg or 4 mm Hg to capture the behavior of the lymphangions subjected to positive and negative axial pressure gradients without appreciably effecting transmural pressure. The inlet and outlet resistances were set equal to the resistance calculated from the bath.

The lymphatic vessel is said to have antegrade propagation when the contractile wave is observed to travel towards the great veins in the neck and retrograde propagation when the contractile wave travels towards the initial lymphatics. By varying the time delays between the lymphangions, the antegrade propagation (positive Δt) and retrograde propagation (negative Δt) of the contractile wave can be simulated.

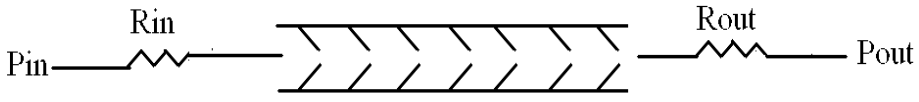

Fig. 19. Model of lymphatic vessel with six lymphangions. R_{in} and R_{out} are input and output resistance. P_{in} and P_{out} are inlet and outlet pressures. Venugopal et al. (26)(© 2003 IEEE)

5.1.2 Model predictions

Instantaneous flow was calculated and averaged from the model of an entire lymphatic vessel as a function of time delay between contractions of adjacent lymphangions (Fig. 20). In all cases, there are two pronounced optimum flows when the delay had small positive or negative values.

When the vessel model was pumping up a pressure gradient ($P_{in} < P_{out}$), a maximum flow of 0.40 ml/s was predicted when the time delay was negative, i.e., when there was retrograde propagation of the contractile wave. However, this is not much different from the local optimum of 0.38 ml/s when the time delay was positive, i.e., when the contractions propagated in the antegrade direction. Similarly, when the axial pressure gradient was reversed ($P_{in} > P_{out}$), a maximum flow of 0.98 ml/s was observed when there was retrograde propagation of the contractile wave. However, this is not much different from the local optimum of 0.90 ml/s when there was antegrade propagation of the contractile wave. These optima occurred when the time delay was quite small (-0.1, 0.3 sec).

A local minimum occurred when the time delay between contractions was zero, i.e., when all lymphangions pumped simultaneously. When pumping under edematous conditions ($P_{in} > P_{out}$), the value of flow was 0.73 ml/s, only slightly less than the optima. However, when pumping up a negative axial pressure gradient ($P_{in} < P_{out}$), flow was 0.06ml/s, a value very close to the flow minima.

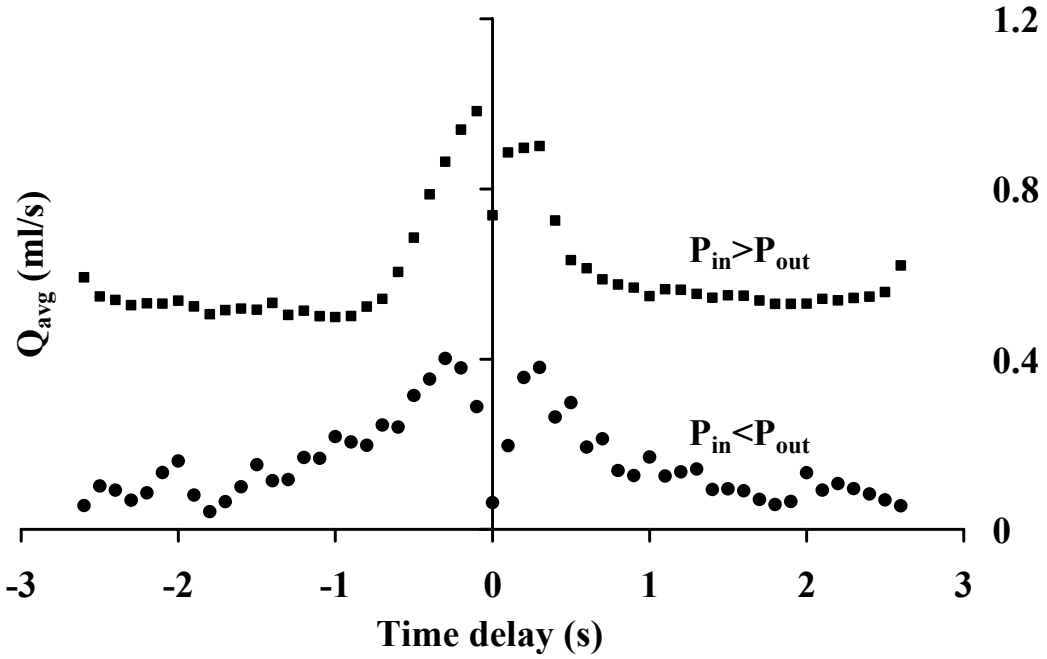

Fig. 20. Model prediction of the effect of time delay on flow. Average flow in lymphatic vessel model shown in Fig. 19 as a function of time delay between lymphangions. Negative time delays indicate that downstream lymphangions contract before upstream lymphangions. When $P_{in}>P_{out}$ (■) and $P_{out}>P_{in}$ (●), maximum flow is observed when the lymphangions pump almost synchronously and the flow is reduced from the optima when the lymphangions pump simultaneously. Venugopal et al.(26) (© 2003 IEEE)

5.2 Effect of frequency on lymph flow

5.2.1 Modeling methods

Lymphangion frequency can have a significant effect on the flow (Figs. 17 and 18). The unique flow pattern is due to the difference in the frequency between the adjacent lymphangions. To evaluate the effect of difference in frequencies on lymph flow, a lymphatic vessel model with two lymphangions is considered. The model is set up such that the lymphangion frequency can be controlled independent of one another.

Lymphangions were made to pump with a set difference in frequency between the first lymphangion (F_1) and the second (F_2). Similar to the time delay, by adjusting the frequency of the time-varying elastance in the model, the contractile frequency is varied. However, the ratio of systolic period to the diastolic period is maintained constant. The instantaneous flow obtained is averaged through an entire cycle to determine the effect of the frequency difference on mean flow (Fig. 21).

The pressure at the inlet was set at 5 mmHg and the outlet pressures was set at either 6 mmHg or 4 mmHg, to make the vessel either pump up a pressure gradient or flow down a gradient, respectively.

5.2.2 Model predictions

The frequency differences between the lymphangions were varied from 0-12 beats per minute and the instantaneous flows calculated were averaged over a cycle. The frequency difference had very little effect on the average flow, even though it

had a significant effect on the pattern of the instantaneous calculated flow (Figs.16 and 17).

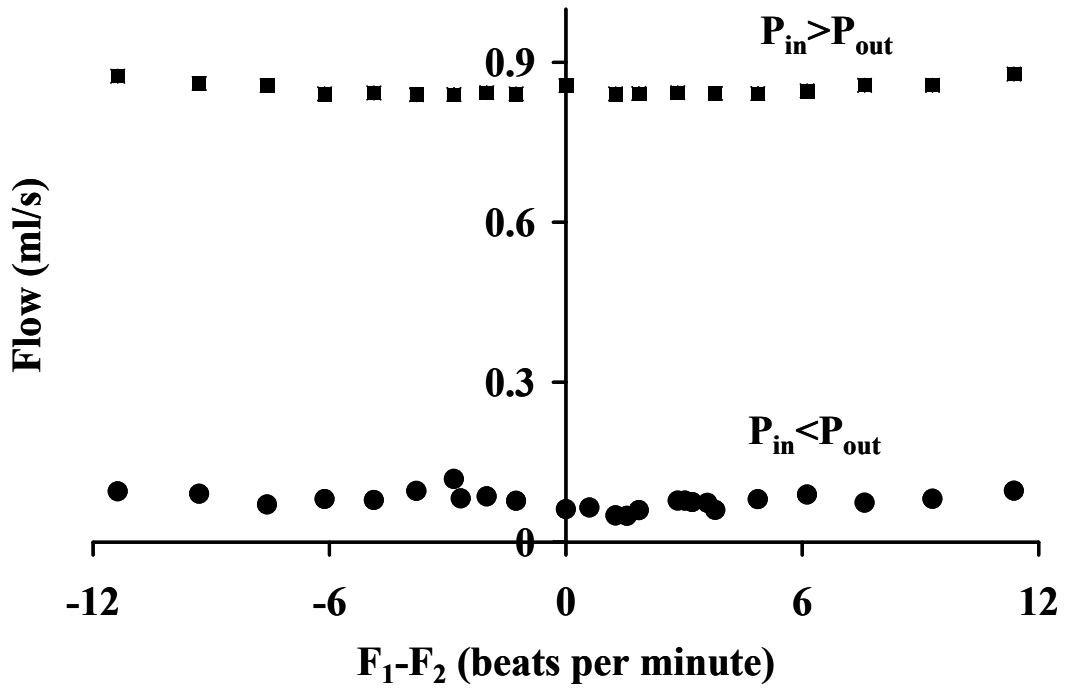

Fig. 21. Model predictions of the effect of difference in frequency between lymphangions on flow. Average flow in lymphatic vessel model as a function of difference in frequency between lymphangions. Negative frequencies indicate that downstream lymphangions have higher contractile frequency than the upstream lymphangions. When $P_{in}>P_{out}$ (■) and $P_{out}>P_{in}$ (●), the flow seems to be not dependant on the frequency difference between the lymphangions Venugopal et al.(24)

CHAPTER VI

DISCUSSION AND CONCLUSION

6.1 Lymphangion model

6.1.1 Validation

A basic model of the lymphangion has been developed based on time-varying elastance concept. This model has been shown to match the flows obtained from the *in vitro* set up for positive and negative axial pressure gradients. When the model is subjected to negative axial pressure gradients, the model predicts that there is flow only during systole, matching the experimental result. However, with a positive axial pressure gradient, there is always a flow from the lymphangion, predicting similar results obtained from the experiments. The amplitude for the flows obtained from the model, however, does not correspond to the values obtained from the experiments, since there can be other vessel properties like elastance, length, and frequency of contraction that affect the particular values of flow.

One major simplification is that the model assumes uniform behavior for all lymphangions. The elastance data used in the model is representative of the numerous elastance curves obtained from the experiment. As we have observed notable variation in the elastance of individual lymphangions, we can expect each lymphangion in the vessel to have different values of maximum elastance (E_{max}),

value of minimum elastance, and possibly the frequency of contraction. The lymphangions which were used in building the model were different from the lymphangions used to validate it.

The model was validated for flows at particular boundary conditions. However, the parameters in the model can have a significant effect on flow. For instance, the amplitudes of the flow can be varied by changing the parameter values of E_{max}, frequency, length, and size. Similarly model predictions may fail under some conditions like high transmural pressure, since the effect of transmural pressure on the lymphangion pump has not been included in the design of the model.

6.1.2 Pump-conduit behavior

The characteristics of the flow are seen to be influenced by the axial pressure gradient. This duality in the nature of flow during these conditions can be characterized using pump/conduit behavior. This duality can again be attributed to their properties of both the heart and the blood vessels. As we can observe from Figs. 10 and 11, there is flow from an independent lymphangion only during the systolic period of the contraction when the lymphangion is subjected to a negative axial pressure gradient. This flow can be attributed to its behavior as a pump, since the pumping of the lymphangions become imperative for the valves to open. However, during positive axial pressure gradients, the valves are predicted to remain open, thus allowing the lymph to flow though them continuously. During these conditions, the lymphangion pumping actually inhibits flow, thus making the system inefficient (25). An interesting physiologic response has been reported by Gashev et al. (8).

They showed that an imposed flow due to positive axial pressure gradients decreased lymphangion contractions, and nitric oxide (NO) was partly responsible for the flow-mediated relaxation. Hence, this relaxation makes the vessel more like a conduit, during which the valve may not close.

This pump-conduit duality is further supported by Figs. 14 and 15, where the flow from the lymphangion is calculated as a function of axial pressure gradients. There is not much of a change in flow with very low values during negative pressure gradients, and there is a linear increase in the flow, with much higher values, when the axial pressure gradient is positive. Furthermore, the hypothesis that lymphangion pumping when subjected to positive axial pressure gradients results in decreased flow is supported by the model predictions and experimental observations reported in Figs. 15 and 16.

6.1.3 Frequency effects on flow

Each lymphangion in a lymphatic vessel acts as an independent pump and can have different frequencies of contraction. There is a significant difference in the observed flow patterns (Figs. 16 and 17) arising from coordinated and dis-coordinated lymphatic vessels.

With this basic model, we now have the ability to consider how lymph transport by the lymphatic system may be affected by lymphangion properties. In particular, this framework allows us to explore how the functional form of the time-varying elastance curve and frequency of contraction may affect the optimal timing of contraction.

6.2 Effect of timing and frequency on lymph flow

The lymphatic vessel model predicts that lymphatic flow is optimized when there is a small time delay between lymphangion contractions (Fig. 19), with very little difference if contraction occurs first in an upstream or downstream lymphangion. This implies that there should be a time delay for an optimal flow and the direction of propagation of contractile wave has little effect for an optimum. This also explains why both antegrade and retrograde propagation of contractile wave observed (2, 29) in lymphangions have similar effects on flow. This indicates that the lymphangion coordination need not be initiated by the mechanical stretch receptors, (resulting in antegrade propagation), but can also be initiated by the electrical connections (resulting in both antegrade and retrograde propagation) which can give similar results.

The model also predicts that the coordination of the lymphangions in the vessel has very little effect on flow. This is illustrated in Fig. 21, where flow remains almost the same when plotted as the function of difference in lymphangion pumping frequency. This means that whenever the lymphangions are totally dis-coordiated, there is very little effect on the mean flow over a period of time, indicating that the lack of connection across the valve (2, 10) need not necessarily be disadvantageous. However, the flows can be affected because the ratio between systole and diastole is maintained a constant.

Because of the inability to independently alter the timing and frequency of lymphangion contraction experimentally, there is no direct evidence to validate the

predictions of the model. This is a case, however, where the complexity of the system is such that computational modeling may be the only feasible way to explore it.

6.3 Assumptions and caveats of the model

The model is based on the time-varying elastance concept. Even though it is the most popularly used model for describing the contractile behavior, there are some concerns over its use. The model assumes a linear end-systolic and end-diastolic pressure-volume relationship. However, the end-diastolic relationship is exponential and the linear end-systolic relationship is yet to be completely characterized for lymphangions. This model also assumes that with increases in chamber volume there is an increase in developed pressure. However, this may not be true if the lymphatic vessels are extended beyond a critical value, beyond which the ability of the lymphangion pump can decrease. This pump failure with an increase in chamber volume cannot be obtained with this model, since the lymphangion model will pump higher volumes for all increases in filling pressure.

By defining the time-varying elastance, the contraction strength and frequency is defined, thus neglecting the effects of transmural pressure on the contractility. The variation in contraction strength can have a significant effect on the amount of volume ejected, and thus the values of flow can be different from one lymphangion to another.

In addition, the contraction cycle is assumed to have the same systolic and diastolic time periods irrespective of other factors. The lymphangions have been very

often shown to have a refractory period (2) between contractions. The elastance used in this model does not have a variable refractory period which can have a significant influence on stroke volume. The internal resistance added to the ventricular elastance described by Shroff (20) has been neglected, and by addition of this resistance to this time-varying elastance, the contractile function may be better described. In the case of the ventricle, this resistance can describe a decrease in chamber volume due to the Hill Effect (14), and in this case, would help describe shear-relaxation.

Despite some conceptual difficulties (14) with the time-varying elastance concept, it has been a useful description of ventricular contraction that is insensitive to both preload and afterload. Even ignoring variation among lymphangions, however, experimental verification of the independence of elastance to preload and afterload has to be validated for lymphangions.

There are other simplifications associated in characterizing the motion of the lymph. The volume was calculated by assuming that the vessel is cylindrical, although there are notable non-uniformities during contraction. Poiseuille's Law was used to describe resistive loss. However, Poiseuille's Law is an approximation for cylindrical rigid tubes. Likewise, our equation to describe inertance was originally developed for blood vessels undergoing small deformations. Since these equations are used in describing the lymphangions, undergoing larger deformations, it can have an effect on flow.

The equations that are used do not describe the effects of shear-induced relaxation. Hence, during high flow conditions, the lymphangion radius is assumed

not to change. However, the resting tone of the lymphangions has been observed to change (8). In addition, there is a decrease in the contraction frequency of the lymphangions observed under high flow conditions, which has not been considered in the model. The absence of this effect can be observed from the Figs. 14 and 15, where the percentage difference between the calcium-free solution and the normal solution is much higher in the experimental data than the model predictions. Notably, the model was made passive without changing the EDPVR.

We used a rather simple fluid dynamic model which includes the effects of viscous losses and the inertia of the lymph. The equation used in describing the resistance (Poiseuille's Law) and the inertance can be derived from Navier-Stokes by assuming cylindrical vessels and small changes in vessel radius. Reddy et al.(15) have used similar equations derived from Navier-Stokes.

The transmission line description used to characterize vessel behavior is a very good model for small vessel segments. An ideal description of a lymphangion would be perfectly distributed. However, in this model all the inertances, resistances and compliances were lumped. This approximation can particularly have an effect when the lymphangion model is simulated with large values for length.

In spite of these limitations, this model has been successful in capturing most of the basic phenomenon observed in contracting lymphatic vessels, and provides basic insight into the role of frequency and timing in lymphangion coordination.

REFERENCES

1. **Benoit JN, Zawieja DC, Goodman AH, and Granger HJ.** Characterization of intact mesenteric lymphatic pump and its responsiveness to acute edemagenic stress. *Am J Physiol* 257: H2059-2069, 1989.

2. **Crowe MJ, von der Weid PY, Brock JA, and Van Helden DF.** Co-ordination of contractile activity in guinea-pig mesenteric lymphatics. *J Physiol* 500 (Pt 1): 235-244, 1997.

3. **Drake R, Giesler M, Laine G, Gabel J, and Hansen T.** Effect of outflow pressure on lung lymph flow in unanesthetized sheep. *J Appl Physiol* 58: 70-76, 1985.

4. **Drake RE, Adcock DK, Scott RL, and Gabel JC.** Effect of outflow pressure upon lymph flow from dog lungs. *Circ Res* 50: 865-869, 1982.

5. **Drake RE, Allen SJ, Katz J, Gabel JC, and Laine GA.** Equivalent circuit technique for lymph flow studies. *Am J Physiol* 251: H1090-1094, 1986.

6. **Drake RE and Gabel JC.** Effect of outflow pressure on intestinal lymph flow in unanesthetized sheep. *Am J Physiol* 260: R668-671, 1991.

7. **Drake RE and Gabel JC.** Effect of outflow pressure on liver lymph flow in unanesthetized sheep. *Am J Physiol* 259: R780-785, 1990.

8. **Gashev AA, Davis MJ, and Zawieja DC.** Inhibition of the active lymph pump by flow in rat mesenteric lymphatics and thoracic duct. *J Physiol* 540: 1023-1037, 2002.

9. **Li B, Silver I, Szalai JP, and Johnston MG.** Pressure-volume relationships in sheep mesenteric lymphatic vessels in situ: response to hypovolemia. *Microvasc Res* 56: 127-138, 1998.

10. **McHale NG and Meharg MK.** Co-ordination of pumping in isolated bovine lymphatic vessels. *J Physiol* 450: 503-512, 1992.

11. **McHale NG and Roddie IC.** The effect of transmural pressure on pumping activity in isolated bovine lymphatic vessels. *J Physiol* 261: 255-269, 1976.

12. **McHale NG, Roddie IC, and Thornbury KD.** Nervous modulation of spontaneous contractions in bovine mesenteric lymphatics. *J Physiol* 309: 461-472, 1980.

13. **Noordergraaf A.** *Cardiovascular System Dynamics*: Academic Press, New York, 1978.

14. **Palladino J, Mulier J, and Noordergraaf A.** Defining ventricular elastance. *Proceedings of the 20th Annual International Conference of the IEEE*, Hong Kong, 1998, p. 383 - 386.

15. **Reddy N and Kesavan S.** Low reynolds number liquid propulsion in contracting tubular segments connected through valves. *Mathl Comput Modelling* 12: 839-884, 1989.

16. **Reddy NP, Krouskop TA, and Newell PH, Jr.** Biomechanics of a lymphatic vessel. *Blood Vessels* 12: 261-278, 1975.

17. **Reddy NP, Krouskop TA, and Newell PH, Jr.** A computer model of the lymphatic system. *Comput Biol Med* 7: 181-197, 1977.

18. **Reddy NP and Patel K.** A mathematical model of flow through the terminal lymphatics. *Med Eng Phys* 17: 134-140, 1995.

19. **Sagawa K, Suga H, Shoukas AA, and Bakalar KM.** End-systolic pressure/volume ratio: a new index of ventricular contractility. *Am J Cardiol* 40: 748-753, 1977.

20. **Shroff SG, Janicki JS, and Weber KT.** Evidence and quantitation of left ventricular systolic resistance. *Am J Physiol* 249: H358-370, 1985.

21. **Suga H and Sagawa K.** Instantaneous pressure-volume relationships and their ratio in the excised, supported canine left ventricle. *Circ Res* 35: 117-126, 1974.

22. **Suga H, Sagawa K, and Shoukas AA.** Load independence of the instantaneous pressure-volume ratio of the canine left ventricle and effects of epinephrine and heart rate on the ratio. *Circ Res* 32: 314-322, 1973.

23. **Sunagawa K, Maughan WL, and Sagawa K.** Effect of regional ischemia on the left ventricular end-systolic pressure-volume relationship of isolated canine hearts. *Circ Res* 52: 170-178, 1983.

24. **Venugopal AM, Stewart RH, Laine GA, Rajagopalan S, and Quick CM.** Dis--coordination of lymphangion pumping in lymphatic vessels. *21st Annual Houston Conference on Biomedical Engineering Research*, Houston. Houston Society for Engineering in Medicine and Biology, 2004, p. 101.

25. **Venugopal AM, Stewart RH, Rajagopalan S, Desai KV, Laine GA, and Quick CM.** Lymphatic pump efficiency. *FASEB J.*, 2004, p. A657.

26. **Venugopal AM, Stewart RH, Rajagopalan S, Zawieja DC, Laine GA, and Quick CM.** Applying the time-varying elastance concept to determine optimum coordination of lymphangion contraction in a lymphatic vessel. *Proceedings of the 25th Annual International Conference of the IEEE*, Cancun, 2003, p. 323-327.

27. **Womersely J.** Method for the calculation of velocity, rate of flow and viscous drag in arteries when the pressure gradient is known. *J Physiol (London)* 127: 553-563, 1955.

28. **Zawieja D, Benoit J, and HJ G.** Coordination of lymphatic pumping. A theoretical model (Abstract). *Federation Proc.*, 1987, p. 1546.

29. **Zawieja DC, Davis KL, Schuster R, Hinds WM, and Granger HJ.** Distribution, propagation, and coordination of contractile activity in lymphatics. *Am J Physiol* 264: H1283-1291, 1993.

VITA

Name:	Arun Madabushi Venugopal
Address:	703 Gaytha Circle, Apt. B, Bryan, Texas – 77801, USA.
Date of birth:	May 27, 1981.
Education:	
2002-2004	Texas A&M University Master of Science Biomedical Engineering, 2004.
1998-2002	S.R.M. Engineering College Madras University Chennai, India. Bachelor of Engineering, Instrumentation & Control Engineering, 2002.