AWARD NUMBER: D01 W81XWH-19-1-0087

TITLE: Models of the Lymphatic System for Improved Therapy

PRINCIPAL INVESTIGATOR: Ghassan, Kassab, PhD

CONTRACTING ORGANIZATION: The California Medical Innovations Institute, Inc.

REPORT DATE: June 2020

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release; distribution is unlimited.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE June 2020	**2. REPORT TYPE** Final	**3. DATES COVERED** 01-MAR-2019-29-FEB-2020
4. TITLE AND SUBTITLE Models of the Lymphatic System for Improved Therapy		**5a. CONTRACT NUMBER** W81XWH1910086
		5b. GRANT NUMBER BA160616
		5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S) Dr. Ghassan Kassab, Ph.D E-Mail: gkassab@calmi2.org		**5d. PROJECT NUMBER**
		5e. TASK NUMBER
		5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) 11107 Roselle Street, Suite 211 San Diego, CA 92121-1206		**8. PERFORMING ORGANIZATION REPORT NUMBER**
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Development Command Fort Detrick, Maryland 21702-5012		**10. SPONSOR/MONITOR'S ACRONYM(S)**
		11. SPONSOR/MONITOR'S REPORT NUMBER(S)
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited		
13. SUPPLEMENTARY NOTES		

14. ABSTRACT

In 2005, there were an estimated 1.6 million limb amputations with a projected rise to 3.6 million by 2050. While most amputations are required due to type II diabetes related tissue injury, the second most frequent etiology is traumatic injury. Military personnel are not only at a higher risk to require an amputation due to injury while on deployment, military veterans have also almost three times a higher risk of developing type II diabetes and hence lower limb disease predisposed to amputation. In trauma or amputation surgery, the lymphatic structures responsible for fluid drainage are disrupted, which can result in permanent or recurring lymph accumulation. **This accumulation, called edema can drastically reduce mobility, prosthetics fit and quality of life of affected patients**. The bio-physical disruptions which create an impedance to flow remain unidentified. The lymphatic system is an extensive network of distensible channels that parallels the vascular systems and drains into veins. The lymph circulation collects and transports excess tissue fluid and extravasated plasma protein, absorbed lipids and other large molecules from the peripheral tissues back to the venous system via the thoracic duct. Despite the importance of the lymphatic system for metastization routes, immune response and fluid homeostasis, it remains largely understudied. For example, the pressure gradient and flow velocities between the drainage areas and collecting vessels remain unclear. Advances in high-performance computing make it now possible to attempt anatomically realistic computational models, where anatomical details and mechanical properties of the lymphatics network are considered. Mathematical models are important tools because experimental approaches to the problem are limited; particularly, deep within the musculature, which cannot be directly observed. By decreasing the lymphatic pressure gradient, we can reduce stasis to restore physiological drainage, which is beneficial for prosthetic fit in amputees.

Our general **objective** was to develop the ***first ever*** anatomically correct and physiologically validated model of the major vessels in the lymphatic system of the limb based on anatomically accurate 3D data in a swine model; one that integrates measured anatomical architecture (**Aim 1**) and mechanical properties of the lymphatic system (**Aim 2**) to create a mathematical model which will then be validated *in vivo* (**Aim 3**). The model will specifically elucidate pressure-flow relation in the lower limb, which can be utilized later for clinically significant lymph drainage studies. Our central **hypothesis** is that anatomical and mechanical properties measured *in vivo*, *in situ* and *ex vivo* to inform and calibrate, will provide a mathematical model to predict flow in variable segments of the lymphatic system. This model serves to integrate the physical and biological determinants of lymphatic flow that clarify the lymphatic flow distribution and provide a therapeutic rationale for potential treatments. Due to lack of anatomical mapping and mechanical characterization of the lymphatic system *in vivo*, the absence of a biophysical model of the lymphatic system has been a critical barrier to progress. Our proposal addresses this barrier and has the potential to advance scientific knowledge in multi-scale (tissue and organ), multi-physics (fluid and solid) modeling and, ultimately, clinical practice in diagnosis and treatment of edema in military and civilian amputees. Accordingly, the three **Specific Aims** were to:

1. Reconstruct the major lymphatic tree anatomy of the limb (> 0.5 mm in diameter) mathematically to integrate the architecture of the lymphatic system *in silico.*

2. Measure the mechanical behavior of major lymphatic vessels including distensibility and stiffness of individual large segments (> 0.5 mm). *We measured pressure and diameter relation in vivo and ex vivo through cannulated lymphatic vessels. Our mathematical model allowed us to predict the constitutive equation of the lymphatic vessels.*

3. Predict the distribution of spatial flow in the limb major lymphatic system as well as the pressure-flow relation. *We calculated predicted flow for each major segment of the lymphatics using the constitutive model from Aim 2 integrated with the anatomy of Aim 1 to validate our model pressure-flow relation in vivo.*

The proposed study established a realistic unprecedented biophysical model of the lymphatics system of the lower extremity to elucidate the relationship between pressure and flow, which can be used to elucidate the mechanisms of edema. This will serve as the foundation for therapeutic application, both in prevention and treatment of outflow congestion post amputation surgery.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF: unclassified			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRMC
a. REPORT Unclassified	**b. ABSTRACT** Unclassified	**c. THIS PAGE** Unclassified	Unclassified	39	**19b. TELEPHONE NUMBER** *(include area code)* 301.619.7035

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

The lymphatic system is a highly complex active system with highly variable structure and function between anatomical sites and between species. Despite the importance of the lymphatic system in health and disease, it remains largely under-studied, especially compared with the circulatory system. The lack of mathematical models developed for the study of its function presents a wealth of opportunities for researchers. Unfortunately, this is hindered by the lack of anatomical and physiological data. The only lymphatic model available is the lumped approach which has oversimplified the problem and cannot address the issues mentioned above. There has also been modelling effort on the components of the lymphatic system; i.e., lymphatic valves. We constructed a mathematical lymphatic model that takes into account the branching pattern, diameters, lengths and mechanical properties of the individual lymphatic vessels. This structural/mechanical model in conjunction with laws of mechanics (conservation of mass and momentum) allowed us to synthesize the pressure-flow relation in each lymphatic segment to that of the entire lymphatic system. This allowed us to predict the pressure/flow distribution throughout the lymphatic network. The predicted pressure-flow relation of lymphatic system was validated with experimental measurements.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Modeling, Amputation, Cancer, Circulation, Surgery

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Major Task 1 – Reconstructed the major lymphatic tree anatomy of porcine upper limb (> 0.5 mm in diameter) mathematically to map the architecture of the limb lymphatic system in silico

- Milestone #1: Consensus on model parameters, target date: 05/31/2019, completion status: 100%
- Milestone #2: Computerized model of upper limb lymphatic tree architecture, target date: 08/31/2019, completion status: 100%

Major Task 2 – Measured the mechanical behavior of major lymphatic vessels including distensibility and stiffness of individual large segments (> 0.5 mm)

- Milestone # 3: Mathematical model based on mechanical properties of vessels, target date: 08/31/2019, completion status: 100%

Major Task 3 – Established a mathematical model of lymphatic flow

- Milestone # 4: Formulate lymphatic flow simulation model, target date: 11/30/2019, completion status: 100%

Major Task 4 – Validated predictive model with bench in situ lymphatic flow measurements

- Milestone # 5: Validate mathematical model of lymphatic system, target date: 02/29/2020, completion status: 100%

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Q1: 03/01/2019-05/31/2019

1) Major activities

- Reached consensus on model parameters
- Initiated in vivo and in vitro imaging studies to visualize lymphatic structure in the swine limb
- Initiated lymphatic structure reconstruction based on in vivo images

2) Specific objectives

The objective for this period were two-fold: 1) reach a consensus on model parameters and 2) initiate reconstruction of the lymphatic structure in the swine limb.

Q2: 06/01/2019-08/31/2019

1) Major activities

- Computerized model of upper limb lymphatic tree architecture
- Measured in situ and in vitro pressure and diameter relation in the vessels.
- Measured in vitro flow-pressure relation in single lymphatic vessel.
- Determined the distribution of lymphatic valves in peripheral lymphatic vessels in limb of pig.
- Derived discretized equations for extensible lymphatic vessels, valves and junctions
- Initiated model assemblage from ex vivo network structure

2) Specific objectives

The objective for this period were two-fold: characterize the relation between pressure and diameter in the lymphatic vessels and produce the governing discretized equations for the vessels, valves and junctions. Both objectives were achieved.

Q3: 09/01/2019-11/31/2019

1) Major activities

- Established the technology to monitor peripheral lymphatic dynamics (pressure, flow, geometry) in limb of pig.
- Determined pulse pressure and flow in single lymphatic vessel in limb of pig.
- Observed the changes of peripheral lymphatic dynamics in response to physical stimulus.
- Developed governing equations for lymphangions, junctions and valves
- Determined model parameters including the pressure-diameter characteristics of the vessels and valve resistance based on experimental data
- Coded the equations and implemented boundary conditions
- Made model scenarios to evaluate modeling results
- Modeled animal lymphatic system from swine data including the length, topology and diameters from animal experiments
- Compared modeling results with analytical solutions when applicable

2) Specific objectives

- The objective for this period were two-fold: to code the governing equations and evaluate modeling results. Both objectives were achieved

Q4: 12/01/2019-02/29/2020

1) Major activities

- Used experimental data from animal experiments for validation of computational data
- Created a model of thoracic duct (TD) with relevant geometrical specifications
- Adjusted number of valves and lymphangions and material properties for TD
- Applied relevant boundary conditions based on experimental pressure and flow data
- Analyzed the computational model based on comparison between pressure gradient computed between the model and obtained from the experiments

2) Specific objectives

- The objective for this period were two-fold: use the computational platform to simulate animal models; assess model based on comparison between model results and animal data

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

On Feb. 21, the Society of Interventional Radiology (SIR) Foundation organized a Research Consensus Panel (RCP) to review knowledge of lymphatics and highlight gaps for future research. Dr. Kassab was invited as a panelist and presented on "Tricuspid Regurgitation and the Thoracic Duct: Novel Observations in Large animals" and discussed the research performed in the present grant. Audience consisting of FDA, NIH, industry and academia were invited. Dr. Kassab invited individuals from the army as well. A white paper will result from the discussion that will be published later in the year.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Not applicable as this is the final quarter

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal *disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).*

- We developed a finite element model for lymphatics flow
- The model can simulate pressure and flow in networks of lymphatic vessels
- The model can be extended to include more healthy and diseased conditions
- We implemented measurements from lymphatic system in our model to validate the results
- We created models based on topology of the lymphatic network

Please see the attached report for more details

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Not applicable

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

None

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

None

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were

these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Not applicable

Significant changes in use or care of vertebrate animals

None

Significant changes in use of biohazards and/or select agents

Not applicable

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

1- Y Dabiri, X Lu, X Guo, B Patel, GW Schmid-Schönbein, and GS Kassab, Simulation of Lymphatic Network Flow Based on Experimental Data: A Finite Element Framework, Prepared.

2- Yaghoub Dabiri, Xiao Lu, Xiaomei Guo, Bhavesh Patel, Ghassan Kassab, Mathematical Modeling of Lymph Flow in a network of Lymphatic Vessels, San Diego, April 4-7, 2020 (conference was cancelled due to COVID-19 outbreak).

3- Lu X, Wang M, Han L, Krieger J, Noblet J, Chambers S, Itkin M, Kassab GS. Morphometry and Lymph Dynamics of Swine Thoracic Duct. Lymphat Res Biol., In Press.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

None

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

See above regarding SIR's CRP.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

We wrote Matlab scripts to simulate the lymphatic dynamics.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

<u>Example:</u>

Name: *Mary Smith*

Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): 1234567
Nearest person month worked: *5*

Contribution to Project: *Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support: *The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: Ghassan Kassab
Project Role: PD/PI
Researcher Identifier (e.g. ORCID ID): https://orcid.org/0000-0001-7126-1484
Nearest person month worked: 2
Contribution to Project: Dr. Kassab has been responsible for the overall direction of the studies.

Name: Xiaomei Guo
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID): https://orcid.org/0000-0002-3155-6429
Nearest person month worked: 4
Contribution to Project: Dr. Guo has performed in vivo and ex vivo measurement of anatomical properties of peripheral lymphatic segments in a swine model.

Name: Yaghoub Dabiri
Project Role: Lead model developer/validation
Researcher Identifier (e.g. ORCID ID): https://orcid.org/0000-0003-4778-7501
Nearest person month worked: 1
Contribution to Project: In-silico model development and comparison with experimental data

Name: Bhavesh Patel
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID): https://orcid.org/0000-0002-0307-262X
Nearest person month worked: 2
Contribution to Project: Dr. Patel has coordinated all activities between animal studies and computational modeling.

Name: Geert Schmid-Schoenbein
Project Role: Consultant
Researcher Identifier (e.g. ORCID ID): https://orcid.org/0000-0002-1803-1521
Nearest person month worked: 0.01
Contribution to Project: Dr. Geert Schmid-Schoenbein has analyzed the experimental data and used mathematical modeling to describe them

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
<u>Organization Name:</u>
<u>Location of Organization: (if foreign location list country)</u>
<u>Partner's contribution to the project</u> (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

Models of the Lymphatic System for Improved Therapy

BA160616

D01 W81XWH-19-1-0087

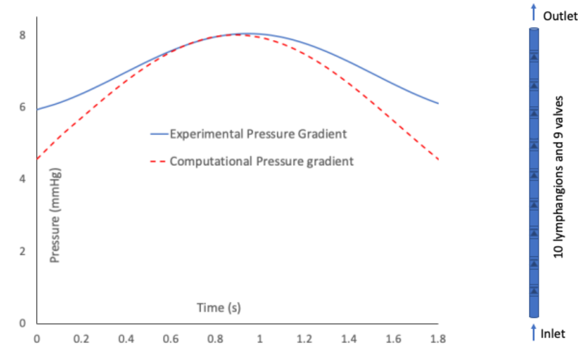
PI: Ghassan Kassab, PhD **Org:** The California Medical Innovations Institute, Inc. **Award Amount:** $336,121

Study/Product Aim(s)

- Reconstruct the major lymphatic tree anatomy of the limb (> 0.5 mm in diameter) mathematically to integrate the architecture of the lymphatic system in silico
- Measure the mechanical behavior of major lymphatic vessels including distensibility and stiffness of individual large segments (> 0.5 mm).
- Predict the distribution of spatial flow in the limb major lymphatic system as well as the pressure-flow relation.

Approach

To achieve the Aims of the study, we will combine in vivo, in situ, bench, and computational approaches using swine as a model. Specifically, lymphatic vessels will be imaged in vivo, flow and pressure values will be measured in vivo, mechanical behaviors of vessels will be characterized in situ and on bench, and all this data will be used to computationally reconstruct the lymphatic structure and simulate associated flow.



Left: The pressure computed by the model and measured experimentally in thoracic duct (TD); Right: Lymphangions and valves were connected in the modeling platform to simulate TD.

Accomplishment: The finite element model was validated against experimental data from TD. The computed pressure was in agreement with experimental pressure.

Timeline and Cost

Activities CY	19			20
Consensus on model parameters				
Computerized model of upper limb lymphatic tree architecture				
Mathematical model based on mechanical properties of vessels				
Formulated lymphatic flow simulation model				
Validated mathematical model of lymphatic system				
Estimated Budget ($336K)	**$84K**	**$84K**	**$84K**	**$84K**

Updated: 03/21/2020

Goals/Milestones

CY19 Goals

– Consensus on model parameters

☑ Determination of model design parameters

– Computerized model of upper limb lymphatic tree architecture

☑Image lymphatic structure in the swine limb

☑Reconstruct lymphatic architecture

– Mathematical model based on mechanical properties of vessels

☑ Passive and active vessel behaviors measured and characterized

– Formulated lymphatic flow simulation model

☑ Network model of lymphatic flow established

CY20 Goal – Validated mathematical model of lymphatic system

☑ Validate model predictions with in situ flow measurements

Comments/Challenges/Issues/Concerns

- None

Budget Expenditure to Date

Projected Expenditure: $336K

Actual Expenditure: $336K

Simulation of Lymphatic Network Flow Based on Experimental Data: A Finite Element Framework

Y Dabiri[1,2], X Lu[2], X Guo[2], B Patel[2], GW Schmid-Schönbein[3], and GS Kassab[2,*]

[1]3DT Holdings LLC, San Diego, CA 92121, USA

[2]California Medical Innovations Institute, San Diego, CA 92121, USA

[3] Department of Bioengineering, University of California San Diego, La Jolla, 92093, California, USA

*Mail correspondence to:

Ghassan S. Kassab, Ph.D.

California Medical Innovations Institute

11107 Roselle

San Diego, CA 92121

gkassab@calmi2.org

Running Title: Lymphatic Network Flow

Keywords: Modeling, Amputation, Cancer, Circulation, Surgery

ABSTRACT

The goal of this paper was to develop a mathematical model of lymphatic system based on measured data of lymphatic anatomy, mechanical properties and boundary conditions. We used finite element method to discretize a one-dimensional continuity and momentum balance equations. In our experimental studies, we visualized the lymphatic network in swine limb in situ, and the pressure drop and flow were measured in lymphatic vessels in vitro. The model includes three types of lymphatic structures: 1) a Lymphangion, 2) a Junction of three or more lymphangions, and 3) Valves. For a lymphangion, the governing equations were nonlinear mass balance and momentum equations including variation in vessel diameter with pressure. In a branch, the pressure did not change and the flow in the outlet vessel was equal to the sum of the inlet vessels. We used lymphatic network topology and boundary conditions from our animal experiments. Results showed that for a lymphangion, pressure drop predicted by the model matches the pressure drop by Poiseuille's law for a rigid vessel. In a branch, the pressure did not change and the flow in the outlet vessel was equal to the sum of flow in inlet vessels. The results for transient boundary conditions showed the model can simulate the transient flow characteristics. The flow simulation results of thoracic duct were in agreement with experimental measurements. The simulation framework in this study provides a tool to analyze flow in lymphatic networks with different topologies under various disease conditions and interventions.

1. INTRODUCTION

The lymphatic system is an important part of the circulation system, which plays an essential role in fluid balance and immune function (Swartz, Hubbell and Reddy, 2008; Jackson, 2009; Choi, Lee and Hong, 2012; Margaris and Black, 2012). Lymphatics regulate the fluid transport and pressure within the tissue, absorption of dietary fat in the intestine and transport of immunological substances. Damage to the lymphatic system leads to lymphoedema, infectious diseases like filariasis, and cardiovascular diseases (Vaqas and Ryan, 2003; Breslin *et al.*, 2018). The roles of the lymphatic dynamics in various diseases is not well understood neither is the lymphatic structure-function relation.

Computational biomechanical modeling of the lymphatic system can be used as a tool to simulate the structure-function relation of this system in health and disease (Reddy, Krouskop and Newell, 1977; Bertram, Macaskill and Moore, 2011; Jamalian *et al.*, 2016). Alterations in the lymphatic structure change the lymphatic flow in a tissue (i.e., structure-function relation). For example, after amputation the lymphatic system is damaged (e.g. by loss of vessels) which alters the mechanics of the lymph flow (Smaropoulos *et al.*, 2005; Lohrmann *et al.*, 2009). In cancer patients the lymphatic system plays an important role in metastatic progression (Alitalo and Detmar, 2012; Padera, Meijer and Munn, 2016).

Experimental studies are limited in providing a full picture of the lymph flow, such as flow velocity and pressure in health, disease or after surgical maneuvers. For example, surgical or interventional maneuvers can be better planned if clinicians can predict the outcomes of a specific procedure using computational models. Hence, validated mathematical models are necessary to better understand the lymphatic system in health and disease (Bertram, Macaskill and Moore, 2011; Margaris and Black, 2012; Moore and Bertram, 2018; Li *et al.*, 2019). To our knowledge, there are currently no computational models that are both informed or calibrated by experimental measurements as well as tested and validated against measurements. Although there are reports on computational simulation of the lymphatic tree, previous studies did not use measured morphometric (e.g., diameter, lengths, branching pattern) data to construct the geometric models nor the lymphodynamic (e.g. flow, pressure) data as boundary conditions.

The goal of this study is to create a framework for the computational simulation of the lymphatic tree using swine subcutaneous lymphatic morphometry (diameters, lengths and connectivity) as well as flow and pressure. In addition, we measured the mechanical properties of individual lymphatic vessels (i.e., pressure-diameter relation). These geometry and mechanical data were used to develop the computational model. Since the governing equations are time- and space-dependent and nonlinear, we used the finite element (FE) method to create the computational results. This method is flexible to allow analysis of different healthy and/or diseased conditions by using new elements. For example, using our framework, it is possible to add branches with different numbers of mother and daughter vessels, or to add elements that represent new or occluded vessels after disease or intervention.

2. METHODS

2.1 Animal experiments

Animal experiments were performed in accordance with national and local ethical guidelines, including the Principles of Laboratory Animal Care, the Guide for the Care and Use of Laboratory Animals and the National Society for Medical Research, and an approved California Medical Innovations Institute IACUC protocol regarding the use of animals in research. Pigs were pre-anesthetized with Telazol (50 mg/ml), Ketamine (25 mg/ml), and Xylazine (25 mg/ml) and maintained with 2% isoflurane. ECG was monitored. Blood pressure cuff was then be placed at appropriate positions. The surgical sites were shaved and cleaned in preparation for catheterization and instrumentation. Venous and arterial accesses were obtained using an introducer sheath placed into the femoral vein and femoral artery. A 3% hydrogen peroxide with 1% dye (Prussian Blue Professional Acrylic Ink; Liquitex Artists Materials, Piscataway, NJ) solution was injected into the skin in the search area. Fine oxygen bubbles produced from the hydrogen peroxide inflated the lymphatic vessel and forced the pigment into the lumen. A small incision was made 2.5 cm proximally from the injection site, and infused lymphatic vessels were identified using a stereo microscope. A homemade micro-catheter (OD: 0.2 mm) was cannulated into the lymphatic vessel and a ligation was applied approximate 1 cm apart the catheter. The catheter was connected to a container filled with saline. The container was lifted step-by-step to desired heights (e.g., 1 cm, 2 cm, 3 cm, etc.) to pressurize the lymphatic vessel. The diameter of the lymphatic vessel was measured at every pressure to obtain pressure-diameter curve of the lymphatic vessel. The limb skin was removed, and the lymphatic vessels were exposed, visualized by the dye and recorded as bright field images.

2.2. Computational Methods

2.2.1 Geometry Reconstruction

From the dye images collected in the animal studies, we visualized the lymphatic vessels by tracing them with the photo editor program "ImageJ" as follows: 1) Major vessels were determined based on diameter of vessels; 2) Junctions between the vessels determined in Step 1 were spotted; 3) Distal points of the vessels open to the capillaries (inlet flows) were determined; 4) Proximal points of the vessels where they connect to largest vessel (outlet flow) were determined; and 5) Judgment about the relative depth of each vessel was based on qualitative evaluation of color intensity (a lighter vessel was considered in a deeper region). This step was used to distinguish junctions from crossings.

2.2.2 Finite Element Model

2.2.2.1 A lymphangion

We assumed flow in each lymphatic vessel was fully developed and one-dimensional. The continuity and momentum balance equations were used to model the lymph flow, as follows:

Continuity:

$$\frac{\partial Q}{\partial x} + \frac{\partial A}{\partial t} = 0 \tag{1}$$

Momentum balance:

$$\frac{\partial Q}{\partial t} + \frac{A}{\rho}\frac{\partial p}{\partial x} + \frac{8\pi\mu}{\rho A} Q = 0 \tag{2}$$

For a rigid vessel, these equations reduce to Poiseuille's law. The lymphatic vessel diameter depends on fluid pressure. To model the alterations in diameter with pressure, we used the results from our animal experiments. A sample diameter-pressure curve is shown in Fig. 1. It should be noted that this data was obtained when active vessel behavior was considered. Thus, it includes both passive and active diameter changes with pressure at steady state.

From this data, the following relation between pressure and diameter was established:

If 0 < P < 7.5 mmHg,

$$D = (\frac{P}{75.0}) \times D_{initial} + D_{initial}$$

If P > 7 mmHg,

$$D = \ D_{initial} + D|_{p=7.5mmHg} \qquad (3)$$

where D is vessel diameter, and P is intralymphatic pressure.

2.2.2.2 Conservation of Mass at Junction

At each junction, it was assumed that the intralymphatic pressure is the same and that the sum of input and output mass is zero. For example, for a junction with two input vessels and one output vessel:

Balance of mass:

$$Q_1 + Q_2 - Q_3 = 0 \qquad (4)$$

Pressure does not change:

$$p_1 = p_2 = p_3 \qquad (5)$$

where Q_1 and Q_2 are the input flows and Q_3 is the output flow; and p_1 and p_2 are the input pressures for the input vessels and p_3 is the pressure at the outflow point of the daughter (outflow) vessel (Fig. 2).

2.2.2.3 Lymphatic Valves

To model valve we used equations suggested in the literature for lymphatic valves, as below (Bertram, Macaskill and Moore, 2011; Jamalian *et al.*, 2016):

$$R_{valve} = R_{vl} + R_{vh}\left(\frac{1}{1+e^{s\Delta p_{valve}}}\right) \qquad (6)$$

where R_{valve} is the resistance imposed by the valve (forward flow). Parameters R_{vl}, R_{vh} and s determine the valve resistance, and Δp_{valve} is the pressure gradient across the valve.

2.2.3 Loads, Boundary Conditions and Nonlinear Solver

There were two degrees of freedom at each node: flow and pressure. For each model, the flow at the inlet nodes and the pressure at the outlet node were enforced as the boundary conditions. This set of boundary conditions led to a system of nonlinear equations for which there was a unique solution (at each time step). To solve this system of equation, an iteration scheme was used. Namely, at the first-time step, the initial values were from a steady-state solution. For subsequent time steps, the unknowns were guessed based on initial conditions, and the linearized system of equations was solved. Then the solution was compared with the guessed values, and if the difference between them was larger than a preselected tolerance, the guesses were updated using the new solution. This process was repeated (at each time step) until the difference between guessed and calculated unknowns were smaller than the tolerance which was set based on flow and pressure values.

In all simulations, lymph was assumed as a Newtonian fluid with density and viscosity as 1000 kg/m^3 and 0.001 Pa.s, respectively. Also, lymph flow was assumed as fully developed given the low Reynold's number of flow. We use Matlab as the programming language.

3. RESULTS

3.1 Experimental Data

The experimental data show the changes in lymphangion diameter with pressure (Fig. 1). The diameter increased with pressure, but it remains nearly constant after pressure reaches a threshold (~0.686 kPa in Fig. 1).

3.2. Computational Results

3.2.1 Scenario 1: Single lymphangion

The lymphangion had a length and initial diameter of 3.5 and 0.36 mm, respectively (Fig. 3), based on reports in the literature (Suami and Schaverien, 2016) and our experimental measurements (Lu et al, 2020). The input flow was assumed to be a second-order curve (Fig. 3); and the outlet pressure was assumed to be 0 kPa.

3.2.2 Scenario 2: Two Lymphangions

In this case, two lymphatic vessels were connected to form a single vessel (Fig. 3). The inlet flow boundary conditions were similar to Scenario 1. The pressure drop was larger than a single lymphangion.

3.2.3 Scenario 3: Two lymphangions in series with a valve between them

Here, the outlet of a lymphangion was connected to the inlet of a valve and the valve outlet was connected to another lymphangion inlet (Fig. 4). Compared to the previous scenarios with no valve, the pressure drop was noticeably increased in the presence of a valve.

3.2.4 Scenario 4: Two lymphangions connected to two valves which are connected to a junction and the junction is connected to a valve and a lymphangion.

This scenario included all three element types: Lymphangion, valve and junction. In comparison to previous scenarios, the pressure drop was larger (Fig. 4). The shape of the pressure curve was similar to a single lymphangion or two lymphangions in series without a junction.

3.2.5 Scenario 5: Geometry of subcutaneous lymphatic network in pig limb.

The network topology, dimensions of the vessels and initial areas were determined from animal experiments (Fig. 5). The dimensions of the vessels are indicated in Table (1). The outlet flow was similar to the inlet flow in shape and nearly twice in magnitude. It was assumed there is one valve in each vessel (Fig. 5).

3.2.6 Scenario 6: Flow in thoracic duct (TD).

Flow in TD was simulated based on animal data from our recent study (Lu et al. 2020). It was assumed there are 10 lymphangion elements, and 9 valve elements. The inlet flow in shown in Fig. 6, and the outlet pressure was assumed to be zero. The resultant computed pressure gradient and experimental gradient pressures are shown in Fig. 6.

4. DISCUSSION

In this study, the lymphatic system was modeled using FE method. Each section of the lymphatic system was modeled using a finite element type. Namely, we used different element types for a lymphangion, a valve, and a junction between more than two vessels. Using this approach makes the model flexible to include other healthy and pathological conditions. For example, we can define a new element type with four input vessels and one output vessel. Or we can define element types for a vessel obstruction caused by a surgical or interventional procedure. This approach has been implemented for arterial system (Porenta, Young and Rogge, 1986; Dabiri, Fatouraee and Katoozian, 2005), but to the best of our knowledge, our study is the first to use a FE 1-D approach for the lymphatic system.

For a rigid vessel model, the pressure gradient calculated by the model for a lymphangion was almost identical to the pressure gradient predicted by Poiseuille's law. In scenario 4, a junction was successfully modeled. Namely, the pressure in the junction did not change with flow passing through the junction, and the flow at the junction outlet vessel was equal to the sum of flow in inlet vessels (i.e., conservation of mass). Thus, the assumptions were successfully implemented in the model. The small values of lymphatic pressure in Figs. 3 and 4 are in line with pressures recorded in small lymphatic vessels (downstream of terminal lymphatic vessels) in rats (Zweifach and Prather, 1975). Also, the simulation results were comparable to experimental data for TD (Fig. 6). The differences between modeling and experimental curves in Fig. 6 are more noticeable in early and later times. These higher differences are likely due to some features in the lymphatic vessels which were not considered in this study such as the transient diameter reductions with step increase in pressure (Von der Weid, 2013) and also the time-dependent behavior of valves functionality.

The exact values of model results were not compared against experimental data of small lymphangion vessels because it is difficult to measure pressure and flow in these vessels. However, the model predictions were based on the measured dimensions of the lymphatics (Fig. 5). Moreover, the dimensions and boundary conditions from experimental data were used in the analysis of TD (Fig. 6). The modeling framework makes it possible to apply it to other lymphatic networks when experimental data are available.

In this study, we assumed a 1-D lymph flow, which is not as detailed as a 3D model to simulate the lymphatic system because a 1-D model does not simulate exact streamlines, spatial distribution of velocity, pressure, and stress. In particular, because valves have a 3D structure, they affect the flow in a complex way which is not captured by a 1-D flow assumption. As such, extending this study to a 3D dimension will provide important further information about lymphatic system. For example, the eddies and radial velocity components in the lymph flow need to be simulated using a 3D model.

The equations used to describe one-dimensional flow in lymphangions and valves can be improved. We used a time-dependent physics for flow in a lymphangion (equations 1 and 2). However, we did not include transient diameter reductions with step increase in pressure, as seen in a myogenic response experiment (Von der Weid, 2013). Also, we assumed the diameter of a lymphatic is determined by its fluid pressure only (equation 3). A change of lumen diameter/cross-section due to movement of the surrounding via tethered issue (muscle movement, skin compressions, arterial pulse etc.) was assumed to be zero. Also, we assumed lymph flow is only in forward direction, and did not consider valve responses to backward flow. Once the relevant one-dimensional equations for these characteristics of lymphangions and valves are developed, they could be integrated to our FE platform (that includes both steady-state and transient components).

The topology of the lymphatic network has important roles in flow characteristics of lymphatic network (Bazigou, Wilson and Moore, 2014). In this study, we used in-situ swine information to build the model. Noninvasive methods can be used to acquire the topology of the vessels; for example, Multi-Detector-Row Computed Tomography has been used in the literature to obtain lymphatic vessels topology (Yamazaki *et al.*, 2013).

The experimental data, used to model lymphatic networks, were based on swine lymphatics. The length, diameter and connectivity between lymphatic tree may be different in humans and they will vary between organs and individuals. Using data from human lymphatics will make predictions of lymph flow in man necessary and the basis for translational considerations.

Conclusions

In this study, we developed a FE model of the lymphatic system to simulate lymphatic pressure and flow in a network of lymphatic vessels. The transient behavior of the lymphatic flow was also captured by the model. The model includes lymphangions, valves and junctions. The model is adjustable to include other physical conditions (e.g., external compression due to muscle activity or changes in boundary conditions due to the venous system). The results were in agreement with analytical solutions as well as experimental data. The results of this study can be used to optimize surgical or interventional procedures when the models are made patient-specific. In particular, using this study, a clinician can simulate alterations in lymph flow after each interventional scenario, and select procedures that optimize lymph flow.

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Table 1: length and diameter of vessels in Fig. 5.

Vessel number	Length mm	Diameter mm
1	77	0.5
2	53	0.3
3	34	0.6

Figure Legends

Fig. 1: the relation between a vessel diameter with fluid pressure. This curve was obtained for active behavior of the lymph vessel.

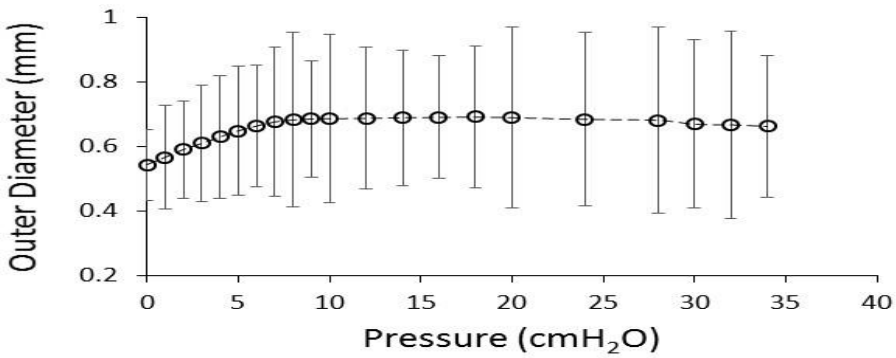
Fig. 2: schematic of lymphatic junction with two input vessels (indexes 1 and 2) and one exit vessel (index 3) and associated valve.

Fig. 3: top: The outlet flow and pressure drop in a single lymphangion; middle: The outlet flow and pressure drop in a model composed of two lymphangions joined together in series; bottom: the inlet flow wave enforced as boundary condition.

Fig. 4: top: the outlet flow and pressure drop calculated for a model with two lymphangions and a valve in between them; bottom: the inlet flow (BC) and outlet flow in a model composed of lymphangions, valves and a branch (scenario 4).

Fig. 5: top: the topology of the model was based on animal experiments; middle: the branch in the left side of the image was simulated by the model; the outlet flow was nearly twice the inlet flow (inlet flow is shown in Fig. 3).

Fig. 6: top: schematic of TD model with five valves; middle: the inlet flow enforced as boundary condition; bottom: the computed inlet pressure in the TD.



Outer Diameter (mm)
1
0.8
0.6
0.4
0.2
0
5
10
15
20
25
30
35
40
Pressure (cmH2O)

Figure 1

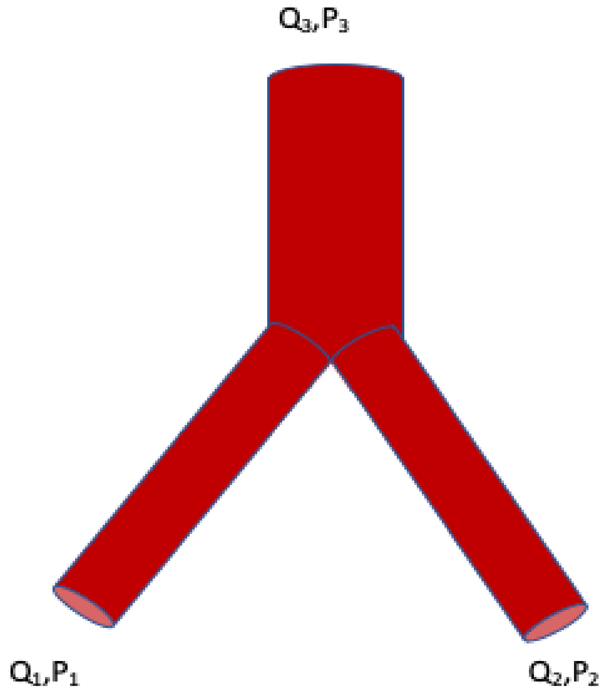
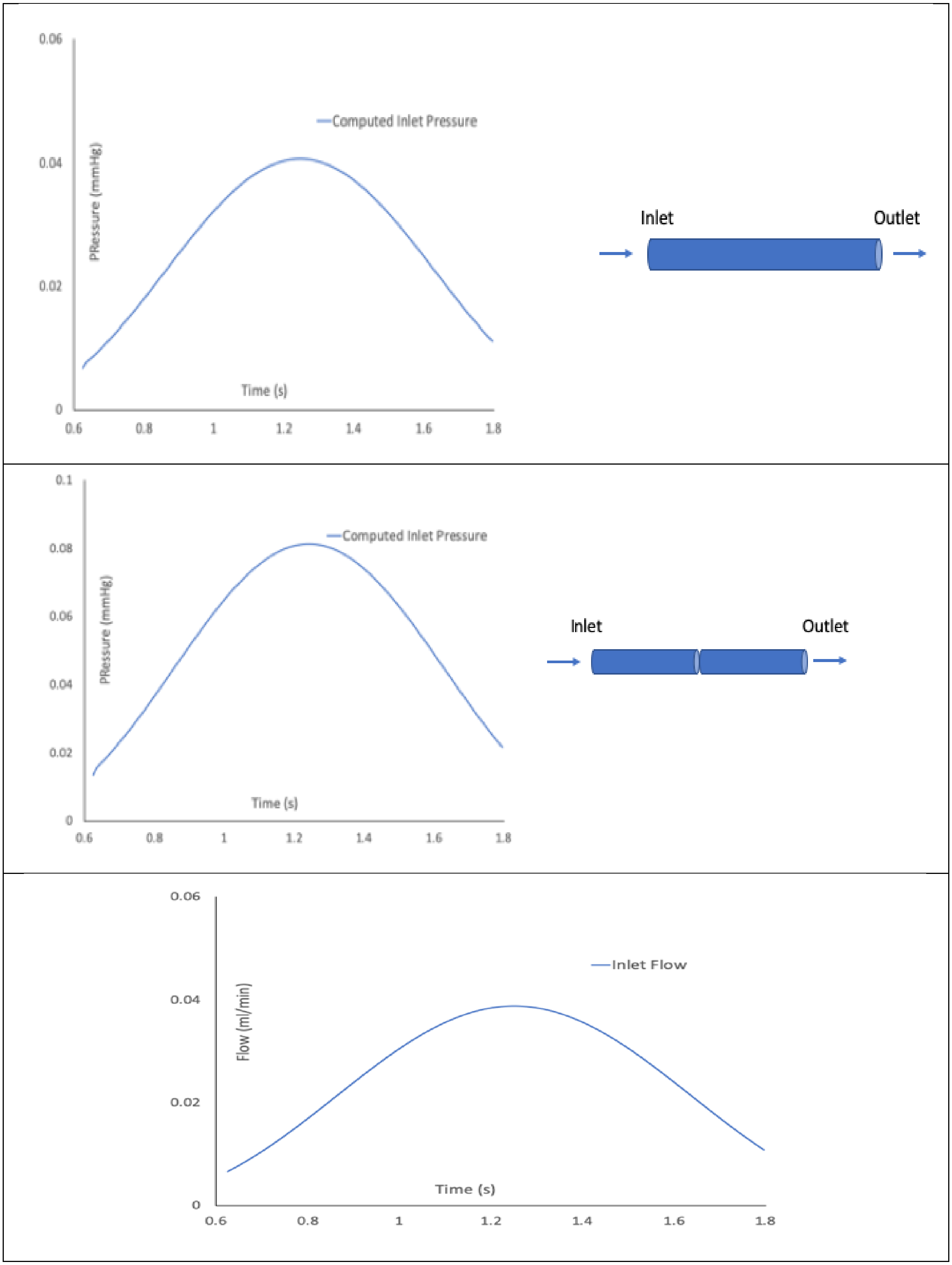


Figure 2



0.06
0.04
0.02
0
Computed Inlet Pressure
PRessure (mmHg)
Time (s)
0.6
0.8
1
1.2
1.4
1.6
1.8
Inlet
Outlet
0.1
0.08
0.06
0.04
0.02
0
Computed Inlet Pressure
PRessure (mmHg)
Time (s)
0.6
0.8
1
1.2
1.4
1.6
1.8
Inlet
Outlet
0.06
0.04
0.02
0
Inlet Flow
Flow (ml/min)
Time (s)
0.6
0.8
1
1.2
1.4
1.6
1.8

Figure 3

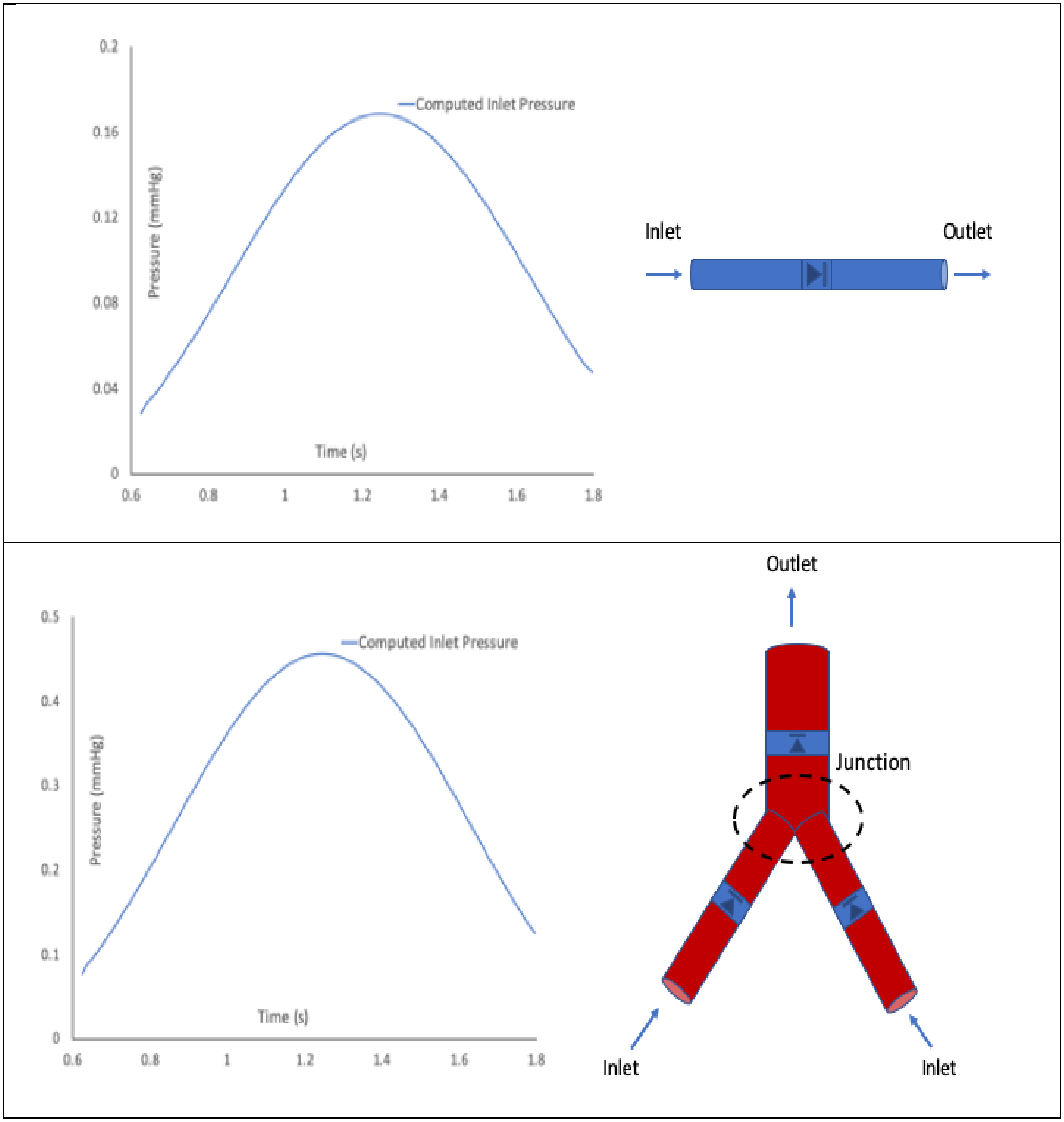
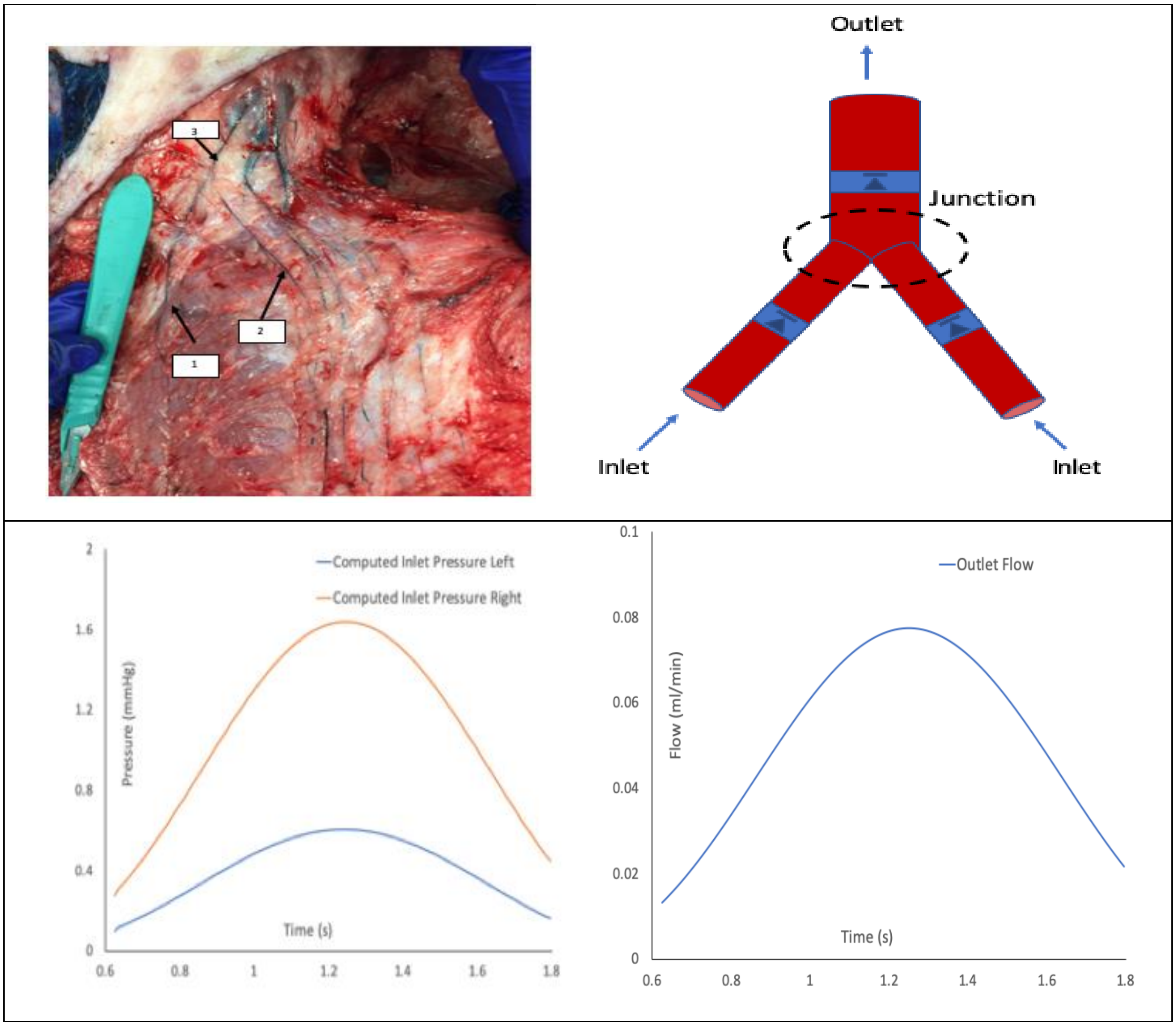
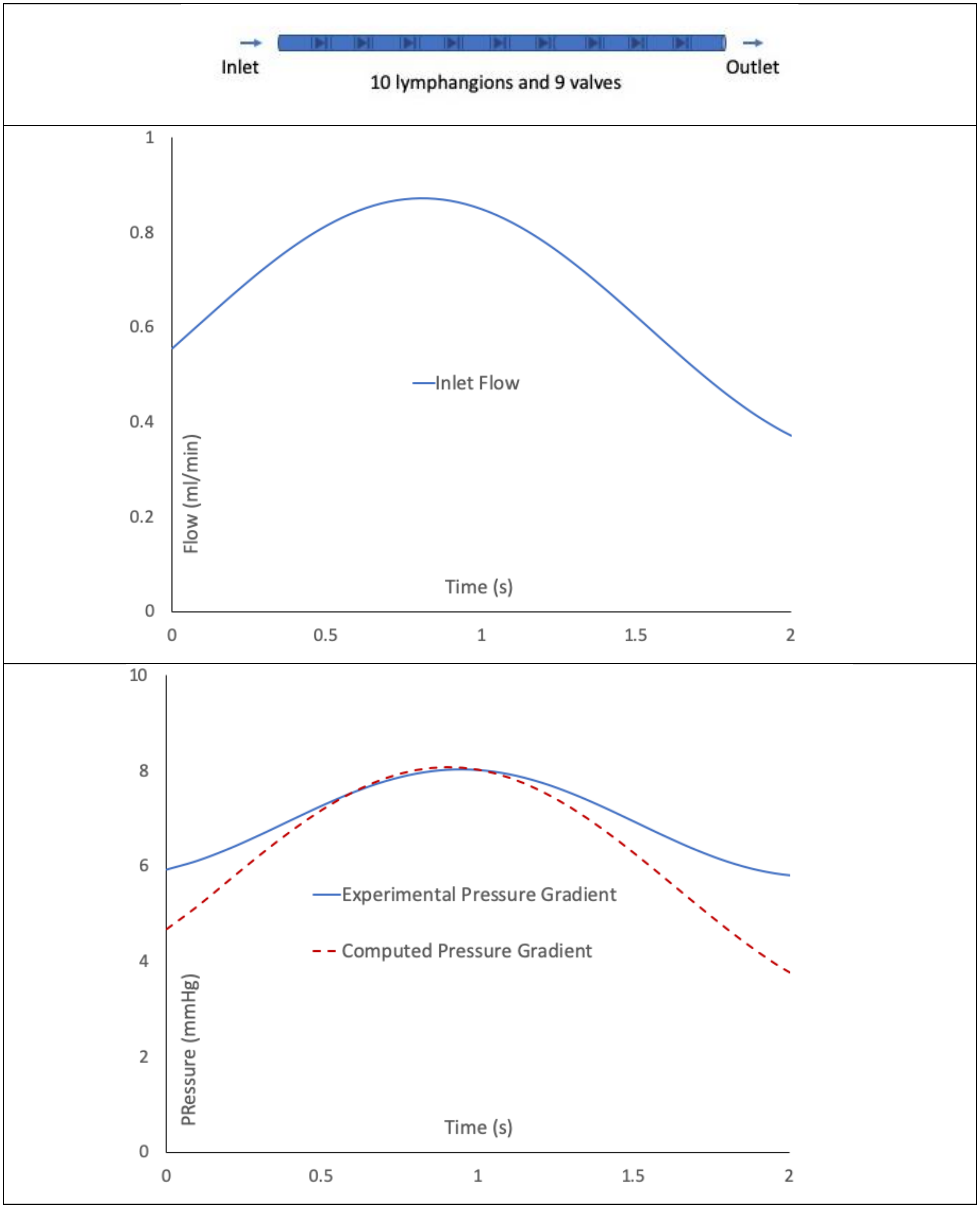

0.2
0.16
0.12
0.08
0.04
0
Pressure (mmHg)
Computed Inlet Pressure
Time (s)
0.6
0.8
1
1.2
1.4
1.6
1.8
Inlet
Outlet
0.5
0.4
0.3
0.2
0.1
0
Pressure (mmHg)
Computed Inlet Pressure
Time (s)
0.6
0.8
1
1.2
1.4
1.6
1.8
Outlet
Junction
Inlet
Inlet

Figure 4



3
2
1
Outlet
Junction
Inlet
Inlet
Computed Inlet Pressure Left
Computed Inlet Pressure Right
Pressure (mmHg)
Time (s)
2
1.6
1.2
0.8
0.4
0
0.6
0.8
1
1.2
1.4
1.6
1.8
Outlet Flow
Flow (ml/min)
Time (s)
0.1
0.08
0.06
0.04
0.02
0
0.6
0.8
1
1.2
1.4
1.6
1.8

Figure 5



Inlet
10 lymphangions and 9 valves
Outlet
1
0.8
0.6
0.4
0.2
0
Inlet Flow
Flow (ml/min)
Time (s)
0
0.5
1
1.5
2
10
8
6
4
2
0
Experimental Pressure Gradient
Computed Pressure Gradient
PRessure (mmHg)
Time (s)
0
0.5
1
1.5
2

Figure 6