Estimation of the Pressure Drop Required for Lymph Flow through Initial Lymphatic Networks

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Abstract

Background: Lymphatic function is critical for maintaining interstitial fluid balance and is linked to multiple pathological conditions. While smooth muscle contractile mechanisms responsible for fluid flow through collecting lymphatic vessels are well studied, how fluid flows into and through initial lymphatic networks remains poorly understood. The objective of this study was to estimate the pressure difference needed for flow through an intact initial lymphatic network.

Methods and Results: Pressure drops were computed for real and theoretical networks with varying branch orders using a segmental Poiseuille flow model. Vessel geometries per branch order were based on measurements from adult Wistar rat mesenteric initial lymphatic networks. For computational predications based on real network geometries and combinations of low or high output velocities (2 mm/s, 4 mm/s) and viscosities (1 cp, 1.5 cp), pressure drops were estimated to range 0.31–2.57 mmHg. The anatomical data for the real networks were also used to create a set of theoretical networks in order to identify possible minimum and maximum pressure drops. The pressure difference range for the theoretical networks was 0.16–3.16 mmHg.

Conclusions: The results support the possibility for suction pressures generated from cyclic smooth muscle contractions of upstream collecting lymphatics being sufficient for fluid flow through an initial lymphatic network.

Introduction

THE LYMPHATIC SYSTEM, WHICH IS CRITICAL for maintaining interstitial fluid balance and is linked to pathological conditions such as cancer progression,¹ consists of two types of vessels: collecting lymphatics and initial lymphatics. During lymph formation, fluid enters the lymphatic system through the initial lymphatic vessels, and it then passes downstream into collecting vessels. These collecting vessels are comprised of a series of lymphangions, which use secondary valves and smooth muscle cell contraction to transport lymph.

In recent years, research has helped elucidate our understanding of the mechanisms involved in regulating this smooth muscle contraction and of fluid movement along these larger contractile vessels.^{2–8} In contrast to collecting lymphatics, initial lymphatic vessels are not wrapped in smooth muscle. With just a single endothelial layer, initial lymphatic function is considered to be passive and remains less understood. Fundamental questions remain unanswered; for example, how

does fluid enter the lymphatic system, and how does fluid move through an initial lymphatic network?

Researchers have recently identified endothelial cell junctional gaps consistent with the concept of one-way primary valves.^{9–11} These findings have provided insight into the passive regulation of fluid flow into initial lymphatics, yet the question regarding how lymph moves through a multi-branched initial lymphatic network requires further investigation. Possible mechanisms include osmotic pressure differences,¹² muscle contractions,¹³ local arterial pulsations,¹⁴ and external compression due to respiring¹⁵ or walking.¹⁶ Given that these hypotheses are either tissue-specific or untested, the potential for each to fully or partially contribute warrants further investigation.

Another possible explanation is the “retrograde pump”¹⁷—the generation of downstream suction pressures due to smooth muscle cell contraction and relaxation in collecting vessels. While this phenomenon has been introduced in the literature,^{18,19} experimental evidence in support of the actual potential for a suction pressure has been limited. Gashev et al. in

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2001 provided direct evidence of negative pressure fluctuations (approximately 2 mmHg) inside an isolated bovine mesenteric contractile lymphatic vessel.²⁰ More recently, Jamalian et al. have provided experimental measurements and computational predictions of negative pressures (up to approximately 2 mmHg) in collecting vessels during the relaxation phase of the pumping cycle.²¹ Based on the values provided by these two studies, a critical question now remains as to whether a few mmHg is a sufficient suction pressure for flow through a real initial lymphatic network.

The objective of this study was to estimate the pressure drop needed for lymph flow through an intact initial lymphatic network. Using a computational approach based on anatomical measurements of intact rat mesenteric initial lymphatic networks, we provide estimates for the potential necessary pressure drops. We additionally report how the required pressure differences might be influenced by different network geometries. Our estimates can be applied to initial lymphatic networks in different tissue types regardless of absolute pressure values and support the phenomenon of the “retrograde pump” as a sufficient mechanism for fluid flow through branched initial lymphatic networks.

Material and Methods

Tissue harvesting and immunohistochemistry

All experiments were performed in accordance with the guidelines of the Tulane University and University of South Florida Animal Care and Use Committees. Adult male Wistar rats (350–400 g) were anesthetized by an intramuscular injection of ketamine (80 mg/kg bw) and xylazine (8 mg/kg bw), then euthanized via 0.2 mL intra-cardiac injection of Beuthanasia (Shering-Plough Animal Health Corp. Union, Kenilworth, NJ).

Mesenteric windows, defined as the thin, translucent connective tissues between the arterial/venous vessels feeding the small intestine, were harvested and placed directly in 10 mM Phosphate Buffered Saline (PBS). Tissues were mounted on positively charged slides, fixed in 100% methanol for 30 min at -20°C , and washed in PBS + 0.1% saponin (Sigma-Aldrich, St. Louis, MO) for 30 min. Tissues were then immunolabeled with primary antibodies and fluorescently labeled secondaries. Lymphatic vessels were labeled with a rabbit polyclonal antibody against lymphatic vessel endothelial hyaluronan receptor 1 (LYVE-1) (AngioBio, Del Mar, CA) and an Alexa Fluor 488-conjugated goat anti-rabbit antibody (Jackson ImmunoResearch).

Imaging

Images were digitally captured by an inverted microscope (Olympus IX70, Olympus America, Inc., Melville, NY) coupled with a Photometrics CoolSNAP EZ camera using a $4\times$ dry objective. $4\times$ montages were used to create a montage for each tissue’s entire area.

Additional images were collected in live tissues using previously described intravital microscopy techniques² with some minor modifications. Briefly, female Sprague Dawley rats (250–275 g) were anesthetized by isoflurane inhalation, a midline laparotomy was performed, and the small intestine and associated mesentery was exteriorized over an optical stage and superfused with Ringer’s solution (Cardinal Health, Dublin,

OH). The mesentery was superfused for 5 min with Ringer’s solution containing $50\text{ }\mu\text{g/mL}$ FITC-BSI-Lectin (Sigma-Aldrich, cat. no. L9381). Images of mesenteric windows were obtained intravitaly and after tissue harvesting and fixation using a Nikon E600 fluorescent microscope system (Nikon Instruments, Melville, NY) and a Photometrics HQ2 camera (Tucson, AZ).

Anatomical measurements and computational modeling

For each sample network ($n=6$ from 5 rats), segment lengths, segment diameters, nodal connections, bifurcation ratios, and network area were measured and recorded. Networks selected for analysis contained a single draining vessel. Segments were assumed to have uniform diameter across length. A node was defined as any point of vessel bifurcation or termination. Branch order one was assigned to the draining lymphatic, and each segment thereafter was assigned a branch order one greater than the segment from which it bifurcates. Bifurcation ratio, B , was calculated per branch order by Eq. 1

$$B_i = \frac{n_{i+1}}{n_i} \quad (\text{Eq. 1})$$

i represents the branch order, and n represents the number of segments at its subscripted branch order. A bifurcation ratio of 2 represents a perfectly bifurcating order, while a bifurcation ratio of 0 represents an order populated entirely by blind ends. Network area was defined as the sum of the area circumscribed around the network being analyzed.

Pressure drops across lymphatic networks were computed using segment lengths, segment diameters, and nodal connections. Segmental flows and nodal pressures were found by solving a system of linear equations considering the conservation of flow at each non-blind node and the pressure-driven Poiseuille flow through each segment, Q (Eq. 2) (Fig. 1).

$$Q = CAP \\ = \left(\frac{\pi D^4}{128\mu L} \right) \Delta P \quad (\text{Eq. 2})$$

C is individual segment conductance, where D represents segment diameter, μ represents lymph viscosity, and L represents segment length. ΔP is the pressure drop due to nodal pressures at either end of the segment. Pressure at blind nodes and flow through the draining lymphatic were treated as known values in the system. All blind nodes were assumed to see the same interstitial pressure (0 mmHg), yet it is important to note that this magnitude is independent of the total ΔP output.

All flow was assumed to enter through the blind ends of the lymphatic network because the objective was to calculate the pressure needed to create flow through the entire network. Nodal pressures were calculated for multiple velocities (2, 4 mm/s)^{22,23} and viscosities (1, 1.5 cp)^{23,24} chosen to represent low and high values reported for flow in a collecting lymphatic vessel, which would be immediately downstream of an initial lymphatic network. The total pressure drop across the network equaled the nodal pressure found at nodal position 1 because all networks had one draining segment and interstitial pressure was set to 0 mmHg.

When calculating the pressure drop across a “subnetwork” up to branch order i , the full network was truncated,

FIG. 1. Example sample network and computational modeling outputs. (A) Mesenteric lymphatic network labeled for LYVE-1. Relative intensity of (B) pressure difference from interstitium and (C) segmental flow, shown by a *jet color spectrum* correlated to a logarithmic scale ranging from the sample's minimum to maximum values. Arrows indicate draining vessel. Scale bar = 1 mm.

considering all segments at order i to be blind and ignoring those beyond it.

Computational prediction of theoretical networks

In order to provide an estimate of potential pressure drop ranges required for networks smaller or larger than the sampled real networks, we created theoretical network geometries. These theoretical networks were modeled using the segment length, segment diameter, and bifurcation ratios measured in the real sample networks and assuming Gaussian distributions. Theoretical networks were capped at 10 branch orders in size. A randomly generated draining vessel bifurcated until network termination, where each segment had the following chance of bifurcation, correlated to ascending branch order: 1.00, 1.00, 0.67, 0.66, 0.60, 0.54, 0.57, 0.49, 0.54, 0.00. These values came from anatomically measured bifurcation ratios divided by 2. Vessels that did not bifurcate became blind ends.

Segment lengths and diameters were randomly generated with a Gaussian distribution about a mean $\pm$ standard deviation and were constrained by anatomically observed minimums and maximums. Length values were distributed about $834 \pm 796 \mu\text{m}$. Diameter values of blind ends were distributed about $30.8 \pm 9.5 \mu\text{m}$, and diameter values of non-blind ends were distributed as follows, correlated to ascending branch order: 61.5 ± 22.6 , 50.4 ± 15.8 , 41.7 ± 12.1 , 42.5 ± 12.1 , 41.0 ± 16.6 , 40.5 ± 15.6 , 37.7 ± 11.0 , 40.4 ± 13.6 , 36.7 ± 12.1 , and $35.2 \pm 11.2 \mu\text{m}$. Diameter values were also constrained by a ratio of segment diameter to parent segment's diameter (0.97 ± 0.31) to ensure a realistic continuity of vessel size. The theoretical networks resulting in the upper and lower 2.5% of calculated pressure drops were discarded as outliers.

Results

Initial lymphatic network geometries

The six lymphatic networks ranged in network area ($12\text{--}129 \text{ mm}^2$), maximum branch order (8–27), and total number of segments (31–219) (Figs. 2; 3A–C). Figure 3D–F

FIG. 2. Initial lymphatic networks. Images of the six mesenteric lymphatic networks used for our analysis were reconstructed in MATLAB after being labeled for LYVE-1. Image reconstructions enables the isolation of individual networks for comparison of network size and branching geometry. Arrows indicate draining vessel. Scale bar = 1 mm.

FIG. 3. Initial lymphatic geometry measurements per sample network. (A) Network area, (B) maximum branch order, and (C) number of segments for each of the six samples. (D) Bifurcation ratio, (E) segment length, and (F) segment diameter plotted against the first 15 branch orders for all samples together and given a logarithmic trend line. Comparisons between first and last branch order were analyzed with a Student's *t*-test with statistical significance accepted for $p < 0.05$. Values are $\pm$ SEM (D–F).

shows the branch order's bifurcation ratio, average segment length, and average segment diameter per network. Segment lengths do not correlate with branch order ($R^2 = 0.199$). In contrast, bifurcation ratios ($R^2 = 0.904$) and segment diameters ($R^2 = 0.827$) display a decreasing logarithmic trend as branch orders become higher and more distal to the draining lymphatic.

Comparing branch order 1 to branch order 15, bifurcation ratio ($p = 5.8 \times 10^{-6}$) and segment diameter ($p = 7.3 \times 10^{-3}$) were significantly decreased. No significant difference between extreme branch orders was observed for segment length ($p = 0.82$). Comparisons between first and last branch order were analyzed with a Student's *t*-test with statistical significance accepted for $p < 0.05$.

Pressure drop estimates across initial lymphatic networks

The range of estimated pressure drops required to move lymph through an entire network was computed to be 0.31–2.57 mmHg for the six sample networks (Fig. 4A). 99% of the pressure drop per full network was accounted for by a smaller subnetwork matching the first eight branch orders (Fig. 4B), indicating that the addition of segments beyond this threshold requires negligible additional pressure. Since a ratio of pressure drops is dependent on the relative resistance values for vessel segments within a branch order versus the entire network, the % of total pressure drop metric can be considered independent of relative flows and viscosity.

In order to ensure a more inclusive pressure drop range that might be characteristic of potential smaller or larger networks and to determine whether networks with altered geometry combinations might influence our extreme estimates, we

created 1000 theoretical network geometries. This number of networks was well beyond the number required for the average pressure drop per network to reach a steady value, identified by the point at which considering n new theoretical networks in addition to n already considered theoretical networks did not perturb the average pressure drop by more than 1%. The estimated pressure value range for the theoretical network geometries was 0.16–3.16 mmHg (Fig. 5).

In this study we used measurements from methanol-fixed tissues, and in order to evaluate the effects of tissue harvesting and fixation on vessel diameter, we conducted an additional experiment. Intravital labeling of rat mesenteric tissues with lectin enabled the comparison of pressurized intra-vital initial lymphatic diameter measurements and post harvesting plus fixation measurements. Fifteen measurements were made along 5 vessel segments. At each of the 15 locations, intravital values were larger versus post fixation values. On average, the intravital measured diameter values were 54% larger than the post fixation values. The maximum and minimum percentage differences were 71% and 35%, respectively.

Increases in diameter values would result in smaller necessary pressure drops as shown in Figure 6, which displays estimates per network for the high velocity, high viscosity inputs. For example, consider network 4. The pressure drop estimate for network 4 using the diameters measured after fixation is 2.57 mmHg. A 20% increase diameter adjustment causes a 31% reduction in pressure drop down to 1.78 mmHg, and a 60% increase diameter adjustment causes a 61% reduction in pressure drop down to 1.00 mmHg.

FIG. 4. Pressure drop estimates across sample networks. **(A)** Pressure differences are computed for four viscosity-velocity combinations. Viscosity constant (μ) set to 1 (low) or 1.5 (high) cp, and velocity constant (v) set to 2 (low) or 4 (high) mm/s. Dotted lines show the overall range of calculated pressures. **(B)** Percentage of total pressure drop incurred by a subnetwork truncated at branch order i . For truncation at branch order i , the pressure drop was computed up to i and compared to the pressure drop computed for the full-sized network. A dashed line shows the threshold at 99%. Branch orders 8 and above minus one standard deviation clear this threshold. Values are $\pm$ SD.

Discussion

The primary contribution of this study is the estimation of the pressure difference required of downstream collecting lymphatics to drive flow through initial lymphatic networks. Our understanding of how fluid flows through initial lymphatic vessels is limited by critical information. Specifically, the potential for a retrograde pump mechanism's involvement is dependent on 1) the demonstration that suction pressures can be generated in collecting lymphatic vessels, and 2) the amount of pressure drop needed for lymph flow through initial lymphatic vessels. Studies by Gashev et al.²⁰ and, more recently, Jamalian et al.²¹ provide support for suction pressures on the order of a few mmHg in collecting contractile lymphatics,

and our results, to our knowledge, provide the first estimates of the required pressure drops for flow through intact initial lymphatic networks. Importantly, our estimate ranges support that the upstream suction pressures might be sufficient for the retrograde pump mechanism.

The rat mesentery tissue was selected for this study because it offers a whole-mount view of intact lymphatic networks (Fig. 1A).^{24,26} The mesentery tissue has historically been used to study initial lymphatic vessel structure and function^{27–29} and is also the most commonly used tissue for investigating contractile mechanisms in collecting lymphatic vessels.^{2,3,5–8} While future studies will be needed to identify whether tissue-specific geometries might influence the pressure drop requirement, it is logical to speculate that our estimates can be generalized to any branched network.

To date, mathematical modeling in the lymphatic system is not nearly as developed as in the blood circulatory system.³⁰ Many models focus on collecting lymphatics, often considering them to be a chain of lymphangions.^{4,31,32} Fewer models consider the initial lymphatics. As examples, work by Mendoza and Schmid-Schönbein focused on flow into a lymphatic vessel across a primary valve,²⁴ and Roose and Swartz used homogenization theory to determine the optimal network formation in terms of fluid drainage.³³

Most relevant to our study, Reddy and Patel created a model to simulate combined fluid absorption and flow through initial lymphatics¹⁹ with a uniform diameter value and uniform length-to-diameter ratio. Their model did not consider a branched network, rather focusing on the interactions between single lymphatic capillaries, permeable walls, and connections with local tissue fibers via anchoring filaments. In the context of these models, the novelty of our modeling is the incorporation of real lymphatic network geometries and the focus on determining the necessary suction pressures for fluid flow.

The computational approach used in our study makes several assumptions. The velocity inputs selected for our study correspond to flow measurements made in downstream mesenteric collecting vessels,^{22,23} as values in initial lymphatics are not well documented, and the viscosity values equal values used for lymph fluid by other groups.^{23,24} The average velocity reported in the Dixon et al. 2006 study²³ is 0.87 mm/s for collecting vessels in the rat mesentery with an average diameter equal to 91 μ m. The average measured diameter for first order vessels in our six networks is approximately 60 μ m. Assuming a direct connection between these first order initial lymphatic vessels and the downstream collecting vessel and considering conservation of mass, for which flow is the same in each vessel, the average output velocity in our networks would be equal to 2 mm/s.

The Dixon et al. 2005 study²² reported a velocity range equal to -1 to 7 mm/s in approximate 100 μ m diameter sized collecting vessels.²² Thus, we consider the 2 mm/s and 4 mm/s velocity values used in our study to be within the physiological range for velocities. However, we do not know if branching occurs in between the draining output initial lymphatic vessel and the collecting vessel. The presence of branches would result in less required flow through our networks. So, the velocity within our networks could be lower than 2 mm/s and our results should be interpreted accordingly. What is important is that the use of these velocities values enable an estimate of the required pressure drop for flow through an initial lymphatic network. While many of our

FIG. 5. Pressure drop estimates across 1000 theoretical network geometries. Each curve represents a network's pressure drop through a subnetwork truncated at branch order i , up to a maximum modeled branch order of 10. The *intensity of shading* represents the distribution of the 1000 pressure curves. The model's minimum and maximum are shown as the pressure curves of the geometries that result in minimum and maximum pressure drops at branch order 10, respectively. The minimum, maximum, and average of the model are represented with *dashed lines*, and the six samples and their average are represented with *solid lines and points* (see legend). Viscosity-velocity pairs used in curve computation are (A) 1 cp, 2 mm/s and (B) 1.5 cp, 4 mm/s.

assumptions remain to be experimentally tested, the results from our study provide numerical estimates for the required pressure drop for flow through branching initial lymphatic networks, and proportionally scaling the calculated pressure drops enables the consideration of altered input parameters (i.e. decreased or increased velocity scenarios).

In addition to viscosity and velocity parameters, network pressure drop estimates are influenced by vessel diameter measurements. Comparison of intravital and post tissue fixation diameter measurements suggests that tissue harvesting and fixation results in smaller vessel diameters. Since resistance for

each vessel segment is inversely proportional to the fourth power of diameter, the diameter effect would result in actual pressure drop requirements being considerably less than those estimated based on the post fixation diameters. Our analysis of the effects of diameter changes on network pressure drop estimates (Fig. 6) accounted for this resistance effect, yet since the output velocity was not changed, the decrease in resistance was countered by an increase in flow proportional to the square power of diameter. Thus, the overall diameter effects seen in Figure 6 only reflect a decrease in pressure drop inversely proportional to the square of the diameter. The pressure drop requirements would be even less for the increased diameter scenarios when the output velocity is decreased to account for conservation of flow.

Our intravital versus post fixation analysis was performed on a sub-set of initial lymphatic segments and additional analysis of vessels per branch order across the hierarchy of networks is necessary to more comprehensively evaluate diameter alteration effects on our pressure drop estimates. Nonetheless, such potential diameter adjustments further support the possibility of a couple of mmHg being sufficient to drive flow through initial lymphatic networks.

A major assumption in our analysis is that initial lymphatic vessels are impermeable. Based on the assumption that primary valves are characterized by overlapping endothelial cell junctions that permit unidirectional flow across the vessel wall,²⁴ lymph fluid would not to leak across the vessel wall into the interstitial space. The lack of leakage in a vessel wall can be modeled as an impermeable wall scenario in which fluid only passes along a vessel. This assumption for primary valves and impermeable walls highlights the limited understanding of how and where fluid enters a network.

Our analysis based on the assumption that fluid enters at blind ends provides a pressure drop estimation for fluid flow through the entire network, but it is conceivable that primary valves at different locations within a network might be open at different times and, not to mention, that evidence for open gaps between endothelial cells²⁸ challenge whether the primary valve paradigm is the only mode for fluid entry.

FIG. 6. Effect of diameter inputs on network pressure drop estimates. Pressure differences are computed for four diameter scenarios. In each case viscosity constant (μ) is set to 1.5 (high) cp and velocity constant (v) is set to 4 (high) mm/s. The measured after fixation scenario corresponds to the estimates in Figure 3A. The 20%, 40%, and 60% larger scenarios represent cases in which the measured diameters have been increased accordingly.

FIG. 7. Example of secondary valves in initial lymphatics. (A) Intact branching network and (B) magnified view of the subsection identified by the dotted box. Arrows indicate secondary valve locations. Secondary valves were identified by morphology and increased label intensity compared to lymphatic segments.⁹ For this image, the tissue was labeled with a mouse monoclonal biotinylated antibody against platelet endothelial cell adhesion molecule (PECAM) (BD Pharmingen, San Diego, CA), streptavidin-peroxidase secondary antibody solution (VECTASTAIN Elite ABC, Vector Laboratories, Burlingame, CA). Images captured with (A) 10 $\times$ dry objective montage and (B) 20 $\times$ oil objective. Scale bar=(A) 1 mm, (B) 50 μ m.

Future investigation is still needed to determine how and where lymph fluid enters an initial network and how these spatiotemporal dynamics might influence pressure gradients and flow distribution throughout a network. Nonetheless, we propose that our assumption of fluid entry at the lymphatic blind ends offers an upper level estimate of the pressure drop. Such a proposition is supported by analysis of laminar flow in cylindrical channels with porous walls, for which trans-wall fluid flow into the lumen reduces the total pressure drop when outflow is conserved.³⁴ Finally, it is important to note that we also assume that all vessel segments are functional and even this scenario necessitates experimental confirmation.

Similar to primary valves at endothelial cell junctions, a void in the literature also exists regarding the presence intraluminal secondary valves in initial lymphatic networks. While PECAM and VE-Cadherin positive secondary valve structures have been previously identified in rat mesenteric networks⁹ the distribution of secondary valves across a network and their function, for that matter, remain to be determined.

As Figure 7 shows, the distribution of apparent valve structures across a network is heterogeneous, with some regions of a branched network having no valves. Since our analysis is only applicable to a scenario with no secondary valves, let us consider the scenario of a network with a one functional valve along a vessel. When the valve is closed along an initial lymphatic vessel with a noncompliant wall, conservation of mass would prevent fluid flow out of that segment. The conservation of mass effect results in a segment just downstream of the valve to the nearest bifurcation and segments upstream of the valve to be, in essence, non-contributors to flow in that portion of the network. This creates a truncated network reducing the needed pressure drop for flow through the equivalent smaller network as supported by Figure 4B.

Interestingly, in the hypothetical scenario of multiple valve closures and no open segments, no fluid would flow out of the network unless vessel walls were compliant. Although our model lacks the incorporation of unknown primary and secondary valve dynamics, our results provide pressure drop estimates in a relevant tissue and represent a realistic scenario that has been modeled by others when the lymphatic pressure exceeds the surrounding fluid pressure.^{10,24}

In summary, this study suggests that flow through sample initial lymphatic networks might only require the generation of up to a few mmHg pressure difference by downstream collecting vessels. Considering this requirement could be even less depending on altered viscosity, velocity, or diameter inputs, our results support downstream smooth muscle cell contraction as a sufficient mechanism for flow. While we still recognize that multiple mechanisms may work together in an additive nature to drive lymph flow,¹⁷ our computational estimates contribute nominal values for the amount of pressure that would be needed by the retrograde pump mechanism.

Such estimates might be especially important for tissue scenarios in which other mechanisms are not present, for example in the mesentery tissue, which is void of external muscle cells and where muscle contraction is not plausible. Our estimates can be applied to initial lymphatic networks in different tissue types regardless of absolute pressure values and serve to reinvigorate discussion and research focused on understanding how flow is driven in initial lymphatic networks.

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Author Disclosure Statement

No competing financial interests exist.

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