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Contraction of collecting lymphatics: organization of pressure-dependent rate for multiple lymphangions

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Abstract

The paper describes the extension of a previously developed model of pressure-dependent contraction rate to the case of multiple lymphangions. Mechanical factors are key modulators of active lymphatic pumping. As part of the evolution of our lumped-parameter model to match experimental findings, we have designed an algorithm whereby the time until the next contraction depends on lymphangion transmural pressure in the contraction just completed. The functional dependence of frequency on pressure is quantitatively matched to isobaric contraction experiments on isolated lymphatic segments. When each of several lymphangions is given this ability, a scheme for their coordination must be instituted to match the observed synchronisation. Accordingly, and in line with an experiment on an isolated lymphatic vessel segment in which we measured contraction sequence and conduction delay, we took the fundamental principle to be that local timing can be overridden by signals to initiate contraction that start in adjacent lymphangions, conducted with a short delay. The scheme leads to retrograde conduction when the lymphangion chain is pumping against an adverse pressure difference, but antegrade conduction when contractions occur with no or a favourable pressure difference. Abolition of these conducted signals leads to chaotic variation of cycle-mean flow-rate from the chain, diastolic duration in each lymphangion, and inter-lymphangion delays. Chaotic rhythm is also seen under other circumstances. Because the model responds to increasing adverse pressure difference by increasing the repetition rate of contractions, it maintains time-average output flow-rate better than one with fixed repetition rate.

1 Introduction

The lymphatic vascular system consists of two main categories of vessel: initial lymphatics, which admit interstitial fluid to become lymph and are non-muscular, and collecting lymphatics, which have muscular walls enabling both tone and relatively quick contractions. The collecting lymphatics are divided into short chambers called lymphangions by one-way

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Conflict of interest

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valves located every 3–10 diameters. In combination, the valves and the contractions enable pumping of lymph, and also lymph suction from the initial lymphatic network upstream (Jamalian et al. 2017). While passive squeezing of lymphatics with intravascular valves also conveys some fluid (extrinsic pumping), particularly in the heart, thorax, gut wall and skeletal muscles (Zawieja 2009), the greater part of fluid and protein transport from the body's interstitial space back to the veins is thought to be achieved by the intrinsic pumping mechanism of lymphatic muscular contraction (Scallan et al. 2016).

In papers since 2011, our group has reported the progressive development and application of a lumped-parameter model of collecting lymphatic vessels. The model, for which equations are given in Appendix 1, has been adapted to situations ranging from emulation of pump limits in an isolated single-lymphangion segment (Bertram et al. 2017), through investigation of optimal pumping in multi-lymphangion chains (Bertram et al. 2016b), to performance prediction for idealised lymphatic networks (Jamalian et al. 2016). The model adheres closely to recent biological observations of lymphatic behaviour, for instance in its treatment of the inter-lymphangion valves (Davis et al. 2011; Bertram et al. 2014a; Bertram et al. 2014b).

Up to now, the timing of the contractions of the individual lymphangions in our multiple-lymphangion model has been organized by two user-adjusted constants: the relaxation time between contractions of a given lymphangion (t_r), and the time delay between contractions of adjacent lymphangions (t_d). A positive value of t_d means that lymphangion 2 starts its contraction a time t_d later than lymphangion 1, and similarly lymphangion 3 later than lymphangion 2, etc., where lymphangion 1 denotes the furthest upstream lymphangion in the modelled chain, according to the flow direction imposed by the one-way valves between lymphangions. Negative values of t_d allow downstream lymphangions to contract before upstream ones. The resulting variations in pumping behaviour were extensively explored by Bertram et al. (2016b).

Since we treat the length of lymphangion systole as fixed, the value of t_r uniquely determines the repetition rate of contractions, and a constant value of t_r means that the repetition rate is unvarying. In contrast, the repetition rate in real collecting lymphatics in a majority of anatomical locations is well known to depend on some measure of the current local distension. This has commonly been expressed through the finding of increased contraction frequency with increased outflow pressure (McGeown et al. 1987); the direct link between contraction frequency and transmural pressure is shown by Gashev et al. (2004) and Davis et al. (2009b), as well as earlier contributors (McHale and Roddie 1976). Although the obvious biological candidate for a locally measurable distension parameter is muscle cell length, Davis et al. (2009a) concluded that in some respects lymphatic myogenic responses relate better to tension, as mediated by pressure, than to stretch.

We have previously incorporated a contraction repetition rate that depends on transmural pressure into a model of an excised lymphatic vascular segment that consists of just one whole lymphangion including its bounding valves (Bertram et al. 2017). Data on contraction frequency vs. transmural pressure were taken from an experiment involving recording of spontaneous twitch contractions at various levels of constant and equal inlet and outlet

pressure from an isolated rat mesenteric collecting lymphatic vessel segment. While it is clear that lymphatic vessels modulate their contraction rate rapidly in response to pressure changes, we decided that it was inappropriate to base the model's varying rate on instantaneous transmural pressure. Instead we computed each successive value of t_f at the end of each lymphangion systole, based on the average of the transmural pressure profile in the lymphangion during that systole. This average was used as input to the measured data on rate vs. pressure, and the frequency so prescribed was used to determine the duration t_f about to begin; see Appendix 1.

However, implementation of a variable contraction rate in a model of multiple lymphangions demands consideration of how the contractions of individual lymphangions are coordinated (McHale and Meharg 1992; Zawieja et al. 1993; Crowe et al. 1997). Simply letting each lymphangion determine its own rate based on its average transmural pressure over the preceding systole would neglect the fact that signals relating to contraction onset are clearly propagated between lymphangions. These signals cause effectively almost simultaneous contraction of contiguous lymphangions under normal circumstances; the timing difference between contractions in adjacent lymphangions is usually less than 0.5 s, and commonly less than 0.25 s (Crowe et al. 1997), or 3% of a typical 8 s contraction cycle. The activation signal can apparently propagate in either direction as readily (Zawieja et al. 1993), and appears to be conducted electrically between muscle cells in the lymphatic wall (Scallan et al. 2016). While the evidence of electrical coupling across valves driving inter-lymphangion coordination is less rigorous than that for electrical coupling between muscle cells within a lymphangion driving the coordinated contraction of the entire lymphangion, Zawieja et al. (1993) found similar conduction speeds in the two situations, suggesting that the mechanism is the same.

In this paper, we describe how we conducted an experiment on an isolated lymphatic vessel to determine principles of contraction coordination. We then incorporated these principles, along with others, in a model of a lymphatic collecting vessel that includes several lymphangions in series. We analyse the rate and pumping responses of the model under a range of pressure boundary conditions, at both realistic and extreme values of the inter-lymphangion conduction rate. We find that the incorporation of variable rate, coordinated according to the principles deduced from the experiment, leads to a more robust performance in the face of varying external challenges than that shown by an equivalent fixed-rate model. Under circumstances where the delay between contractions of adjacent lymphangions is less than the current limit set by inter-lymphangion conduction (as can happen in a variety of circumstances, for instance when that limit is artificially extended or when the lymphangion chain is subjected to low up- and downstream pressures), the flow-rate and other markers of pump performance all show evidence of dynamical chaos.

2 Methods

2.1 Animal experiment

A detailed description of the methods employed is already available (Davis et al. 2011), so only a brief summary will be given here. Animal protocols were approved by the

institutional Animal Care and Use Committee at the University of Missouri, and in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

A collecting lymphatic vessel of diameter some 140–160 μm when not contracting was dissected from the mesentery of an anaesthetized male Sprague-Dawley rat. A segment of the vessel consisting of two complete lymphangions and three valves was cleaned of adherent external tissue and mounted on glass micropipettes via the cut part-lymphangions beyond the first and third valves, all whilst immersed in physiological saline solution. With the pipettes connected to reservoirs supplying an identical pressure of ~ 13 cmH_2O upstream and downstream, the distance between micropipettes was adjusted such that vessel buckling was avoided. The preparation was moved to the stage of an inverted microscope, from which vessel images were recorded continuously at 50 fr/s during the experiment by means of a 2 Mpx monochrome CCD camera (Basler A641FM), interfaced by Firewire to a computer. Inside vessel diameter at a chosen axial location was tracked continuously using a custom algorithm (Davis 2005). Control of the reservoir pressure applied to each micropipette was effected by one of two channels of a microfluidic pressure controller (Elveflow OB1 MK3, Paris). The systems allowed precise independent control according to computer-supplied signals of the pressures p_{in} and p_{out} in the inlet and outlet reservoirs respectively. Arbitrary combinations of step changes could be created for one or both pressures. The pressures p_{in} and p_{out} were transduced by CyberSense 104 transducers (range ± 60 cmH_2O); pressure signals were digitized at 30 Hz via a 16-bit A/D card. The fluid in the perfusing bath applied an external pressure of 0.5 cmH_2O , so transmural pressure p_{tm} was 0.5 cmH_2O less than intraluminal pressure. Flow-rate through the vessel was measured using a Sensirion/Elveflow MFS2 flowmeter (range ± 7 $\mu\text{L}/\text{min}$), which sensed temperature differential in a tube of diameter 150 μm and length 60 mm, upstream from the vessel. The flow detection response time was 40 ms, and the flowmeter was accurate to 5% of the measured value down to 0.4 $\mu\text{L}/\text{min}$, and to 0.02 $\mu\text{L}/\text{min}$ in the range ± 0.4 $\mu\text{L}/\text{min}$ (manufacturer's data). The flowmeter calibration was checked using a Harvard syringe pump and Drummond calibrated microlitre glass tubing. Our tests showed that it gave a reliable and repeatable output at least down to 0.2 $\mu\text{L}/\text{min}$.

After cannulation, but before the bath temperature was raised to 37°C, the vessel was passive. The vessel was equilibrated for 30–60 min at 37°C and $p_{\text{in}} = p_{\text{out}} = 3$ cmH_2O until a stable pattern of spontaneous contractions developed. Then steps of p_{out} were applied, followed by steps of p_{in} . Three steps of p_{out} , taking the pressure to 7, then 11, then 15 cmH_2O , then back to 3 cmH_2O , were applied; each step lasted about 45 s. The steps of p_{in} took the pressure to 5, then 7, then 9 cmH_2O , then back to 3 cmH_2O ; each step lasted about 60 s. Because of the hydraulic resistance of the flowmeter capillary, and that of the two cannulating micropipettes, the pressures applied to the ends of the vessel were not equal to p_{in} and p_{out} when there was flow, i.e. during the steps of p_{in} . Rather the pressure applied to the upstream end would have been less than p_{in} , and that applied to the downstream end, greater than p_{out} . At the maximum mean flow-rate of 4.75 $\mu\text{L}/\text{min}$ reached in the experiment, when the nominal excess of p_{in} over p_{out} was 6 cmH_2O , the flowmeter capillary would have created a pressure drop of some 2.75 cmH_2O .

In subsequent replay, the position of each valve was detected by measuring image intensity change in a narrow window centred on where the valve leaflets pressed together when closed; see Davis et al. (2011) for more detail. The analogue output from the optical densitometer, after normalisation to compensate for varying average vessel-lumen pixel intensity with contraction, was converted to a binary trace by thresholding. The video record was analysed, again by the method of Davis (2005), to determine the time-varying internal diameter at three roughly equi-spaced sites along the vessel (two in lymphangion 1, the third in lymphangion 2); see the image at the top of Figure 1 for the locations. Despite the frame rate, the actual acquisition rate of unique $D(t)$ time-points was 30 Hz. The digitized traces of diameter vs. time were then further analysed to determine times of contraction start at each site; see Figure 2. These times allowed calculation of the inter-site delays and the inter-contraction intervals.

The method of analysis to determine contraction start times was necessarily a compromise between the wish to locate each start as accurately as possible and the practical need to reduce the effects of noise. Best results were obtained by first differentiating the diameter trace, then low-pass filtering the result using a moving-average filter, then applying a negative-going negative-threshold detector. Further manual intervention was needed to deal with trace locations of particular difficulty.

2.2 Mathematical model – coordination of lymphangions

We investigated two different implementations of a control scheme for the timing of lymphangion contractions in the model. Both incorporated concepts of activation signals being conducted between adjacent lymphangions, following precepts that were derived from experimental results (see below). The first involved storing information over all time-points about the three possible lymphangion states (refractory, relaxed, contracting) and the instants of end-diastole and end-systole. Since we preferred to avoid the creation of a large array to hold this information, a second scheme was devised, which tracked these quantities only in the moving time window where they were needed. The scheme encapsulated the following rules:

- any lymphangion can initiate contractions, at a rate which depends nonlinearly on the local pressure, as in our single-lymphangion model (Bertram et al. 2017)
- activation signals commanding the start of contraction are propagated to adjacent lymphangions,
- the propagation time between lymphangions is an adjustable constant (t_p),
- during a refractory period which begins at contraction start and ends after contraction end, a further contraction cannot be provoked, and arriving activation signals are extinguished,
- the cut part-lymphangions beyond the outermost valves in the chain participate in the signal generation and propagation, but do not contract.

As a consequence of the first of these rules, if there is no incoming activation signal, a lymphangion will continue to mount contractions at a rate determined by the local pressure. The inter-lymphangion propagation time (t_p) plays much the same role as the inter-

lymphangion contraction delay (t_d) in our previous model, but as will be seen, delays other than the fixed value t_p can be manifested here.

The numerical scheme which implemented these rules is described in Appendix 2. As a test of the scheme, a pseudo-multi-lymphangion set of slowly time-varying pressures was set up. Each pseudo-lymphangion was characterised by only this one pressure variable; there was no mechanical contraction, and no representation of lymphangion diameter or flow-rate. The assigned pressure in each pseudo-lymphangion varied slowly with time as a linear ramp. The slope of this ramp varied along the lymphangion chain, being greatest for the part-lymphangion before the first valve, and zero for the part-lymphangion after the last valve, and the initial conditions were arranged such that at early time pressure increased along the lymphangion chain, but later it evolved to an increasingly steep decline along the chain; see Figure 3. The plotted quantity of each curve in the second panel is the activation (ranging between 0 and 1). In the full pumping model this waveform controls how the pressure/diameter relationship varies smoothly from the curve which applies when the vessel is passive to that which applies at the peak of contraction, and back again; see Bertram et al. (2017) for further details.

The test proved that the scheme successfully combined the effects of local pressure and inter-lymphangion propagation. At the start, each lymphangion measured its local transmural pressure, and determined a relaxation time accordingly before a contraction would be mounted. Since the last lymphangion (actually the part-lymphangion beyond the last lymphangion) was experiencing the highest pressure at this time, it reached the end of its relaxation period first, and signalled a contraction. For the part-lymphangion, this signal did not cause contraction, but put this element into its refractory state and was propagated to the adjacent (last) complete lymphangion in a time t_p , where it did initiate a contraction. Simultaneously with the start of this contraction, activation signals were propagated to both adjacent lymphangions, again arriving after a time t_p . That propagated downstream, i.e. back to the final part-lymphangion, arrived while this element was in its refractory state, and the signal had no effect and was extinguished. That propagated upstream, from L5 to L4, duly initiated a contraction there that was delayed relative to that in L5 by time t_p . In turn, L4 sent signals to L5 and L3; that to L5 was extinguished, while that to L3 initiated a contraction there, now delayed relative to L5 by time $2t_p$. In this way, a wave of activation progressively spread upstream along the chain, with each lymphangion's contraction delayed relative to its downstream neighbour by t_p . For each lymphangion, the incoming activation signal arrived before the end of its locally determined relaxation time, and caused its next contraction to be brought forward in time.

Subsequently, the progressive reduction of the pressure difference between lymphangions caused diminishing differences between the locally calculated values of t_r . In due course (from about $t = 14$ s), downstream lymphangions reached the end of their local t_r before the arrival of a conducted activation signal, and initiated their own contractions. This caused reductions in both the inter-lymphangion delay and the duration of diastole. The cross-over in lymphangion pressures occurred at 15 s, but not until 25 s did these processes result in all lymphangions contracting simultaneously. The steady further increase in upstream pressures brought about a full reversal of the direction of activation signal propagation, with inter-

lymphangion activation delay reaching the maximum value (t_p) at about 36 s. Thereafter, the sequencing of beats remained fixed, but the duration of diastole continued to decrease as the pressure mounted still further.

After this successful test of the algorithm, equivalent code was then installed in the latest version of our multi-lymphangion model. This is essentially a multiple-lymphangion version of the single-lymphangion model described by Bertram et al. (2017). Like that model, it incorporates measurements of active contractile and passive elastic properties, and the dependence of contraction frequency on transmural pressure, all taken from isobaric twitch contraction experiments in the same vessel. It also models contraction as a progressive warping of the curve of fully relaxed $p_{tm}(D)$ across to meet the curve of maximally contracted $p_{tm}(D)$ and back again, following a prescribed smooth systolic time-course.

We now report on trials of this model, with five lymphangions specified. The fixed parameters of the study were the lymphangion length $L = 0.3$ cm, the lymph viscosity $\mu = 1$ cP, the external pressure $p_e = 1.75$ cmH₂O, the resistances R_a upstream and R_b downstream of the lymphangion chain (these represent the micropipettes in an equivalent isolated-segment experiment), both set to 5×10^6 dyn s cm⁻⁵, the minimum valve resistance $R_{Vn} = 8 \times 10^6$ dyn s cm⁻⁵, the maximum valve resistance $R_{Vn} + R_{Vx} = 10^{10}$ dyn s cm⁻⁵, the valve-resistance-change slope factor $s_0 = 0.2$ cm²/dyn, the valve-closing-threshold attenuation factor $c_{fact} = 0.221$, and the passive and active curves of $D(p_{tm})$, where $D(t)$ is lymphangion diameter and $p_{tm}(t)$ is the transmural pressure $p_m(t) - p_e$, p_m being the mid-lymphangion internal pressure; see Appendix 1 for further explanation of these parameters and variables. The curves of $D(p_{tm})$ were as shown in fig. 1B of Bertram et al. (2017). The relation between t_i and the mean value of p_{tm} over the preceding systole is described in Appendix 1, and was illustrated in fig. 2 of Bertram et al. (2017); Appendix 1 also gives the equations of the multi-lymphangion model. The valve characteristics, which include transmural-pressure-dependent bias to the open state and hysteresis, were as described by Bertram et al. (2014b). With systole taking a fixed 2 s to complete, the refractory period t_n was set to 2.5 s, i.e. lymphangions remained refractory until 0.5 s after the end of each systole, and the parameter m which multiplies the rate of onset and decay of the active state relative to the usual sinusoid (Bertram et al. 2017) was set to 1. In every case the model was started with a simultaneous contraction of all lymphangions, and allowed to find its way to an eventual stationary state involving similar mean flow-rate output from all lymphangions, with that mean evaluated over multiple cycles for instances of irregular rhythm. In cases in which the initial conditions led to a prolonged transient, finding such a stationary state involved integration over many simulated cycles of contraction (up to 300 s of simulated time, with the time-step set in the range 0.0005 to 0.0025 s). As in a past paper (Bertram et al. 2016a), we report values of the mechanical efficiency of pumping, which is the ratio of output pressure-difference/flow-rate work done $\bar{Q}(p_b - p_a)T$ to the work done on the fluid by muscle-derived active tension, $\sum_i \left(0.5\pi l \oint D_i \Delta p_{tm,i} dD_i \right)$, where $\bar{Q}$ is cycle-mean flow-rate, $p_b - p_a$ is the excess of outlet pressure over inlet, and T is cycle time.

3 Results

3.1 Animal experiment

The rat mesenteric lymphatic vessel mostly maintained coordinated contractions along its length. Because of the intra-lymphatic valves, the two step procedures shown in Fig. 1 had different effects. Steps of p_{outlet} above the baseline level of p_{inlet} and p_{outlet} (left side of Fig. 1) caused the segment to continue to pump small quantities of perfusate from inlet to outlet, with all three valves operating cyclically during steps 1 and 2. The mean flow-rates so produced were small, indeed probably too small to register relative to possible zero offset in the flowmeter at the largest value of p_{outlet} . The measured values were 0.17 $\mu\text{L}/\text{min}$ before the first step, 0.06, 0.03, and -0.01 $\mu\text{L}/\text{min}$ during the first, second and third steps respectively, and 0.14 $\mu\text{L}/\text{min}$ after the third step. During step 3, the segment could no longer generate sufficient pressure to open the outlet valve, and the negative value for the third step may reflect either zero offset or genuine leakback. Steps of p_{inlet} above p_{outlet} (right side of Fig. 1), on the other hand, caused a flow-rate through the vessel segment that was much greater than the self-pumped level. In the same order as before, the measured values were 0.15, 1.61, 3.17, 4.75 and 0.16 $\mu\text{L}/\text{min}$. Despite the elevated flow-rates, the repetition rate of contractions did not reduce in this vessel, i.e. shear-related contraction inhibition was not manifested; see Figure S1 in the supplementary material (Online Resource 1). Instead, the repetition rate increased substantially at the higher p_{inlet} -step pressures (also see diameter traces in Fig. 1), as for the p_{outlet} steps, where the mean ID3 inter-beat interval (see Fig. 2) decreased from 6.76 ± 0.14 s (mean $\pm$ s.e.m.) before the steps to 4.96 ± 0.13 s during the first step, 3.80 ± 0.12 s during the second, and 3.24 ± 0.13 s during the third. Only when the stepped pressure reverted to baseline was there a temporary lengthening of the interval between contractions, for both p_{inlet} steps and p_{outlet} steps.

When the reservoir pressures p_{inlet} and p_{outlet} were the same (3 cmH_2O), contractions of the 2-lymphangion segment began approximately 0.3 s later at measurement site 2 than at measurement site 1, and approximately 0.15 s later again at site 3; see Figure 4. This pattern persisted when p_{inlet} was raised above p_{outlet} (right panels). However, when p_{outlet} was raised above p_{inlet} , the time delays became negative, i.e. contraction began soonest at site 3, then spread in a retrograde direction, i.e. opposite to the direction of lymph flow. When p_{outlet} reverted to its baseline value, the retrograde conduction of each contraction immediately reverted to antegrade. Interestingly, whereas conduction from D1 to D2 sped up when retrograde, i.e. the absolute value of the contraction-start time difference was less on average, retrograde conduction from D2 to D3 was slower than antegrade.

3.2 Model outcomes – variation of t_p

With the pump boundary conditions set up for a typical adverse pressure difference between inlet ($p_a = 3$ cmH_2O) and outlet ($p_b = 7$ cmH_2O), and the inter-lymphangion propagation time set to $t_p = 0.3$ s, the five-lymphangion model settled into the contraction sequence 5, 4, 3, 2, 1; see Figure 5. After being started with a simultaneous contraction of all lymphangions, the state of the model evolved towards a steadily maintained pumping condition in which contraction signals propagated upstream along the lymphangion chain. This largely but not entirely reflected the pattern of mean pressures in the lymphangions, as

shown in Figure 6. After 20 s, valve V5 (between lymphangions 4 and 5) stayed open, as can be seen both in Fig. 5 and from the lack of a step up in mean pressure at that location in Fig. 6. This caused the inter-lymphangion delay between lymphangions 4 and 5, which just after 20 s briefly reached 0.3 s as for the other delays, subsequently to evolve to a final value somewhat less than that; see Figure 7. The interpretation of this result is that lymphangion 4 reached the end of its locally determined t_r less than 0.3 s after lymphangion 5 began its contraction, and so initiated its own contraction without waiting for the arrival of the conducted signal. All other lymphangions took their cue from lymphangion 4, each one after a delay of 0.3 s relative to its downstream neighbour. Overall, the response is the attainment of a steady pumping condition after a start-up transient which is only fully complete after some 45 s. During the transient, the cycle-mean flow-rates through valves 1 to 6 differ, reflecting the differing time-courses of instantaneous diameter for lymphangions 1 to 5 as seen in Fig. 5. The final mean flow-rate achieved was 79.3 $\mu\text{L/hr}$, and the mechanical efficiency of pumping was 43.5 %.

We then investigated how the pumping response changed when t_p was set to zero, so that all lymphangions were obliged to contract simultaneously. For the sake of brevity this result is not illustrated, but after a much shorter start-up transient, a steady pumping condition with diastolic duration of 1.125 s in all lymphangions and a mean flow-rate of 49.4 $\mu\text{L/hr}$ was reached. As expected for the case of simultaneous contraction [see Bertram et al. (2016b)], all intermediate valves stayed open, so that one long pump chamber was created. Because valve resistance was thereby minimised, the mechanical efficiency for the chain rose to 58.2 %.

We next investigated the other extreme: absence of conducted activation, with each lymphangion organizing its relaxation period on the basis of its own pressure sensing. This was achieved by setting $t_p = 100$ s, a value much greater than any actual inter-lymphangion delay. As shown in Figure 8, the result was chaotic¹ dynamics, with diastolic duration, inter-lymphangion delay, and cycle-mean flow-rate all varying irregularly from one cycle to the next. A ‘cycle’ is here taken as the time from the start of one contraction to the start of the next, and thus the duration varies from one cycle to the next and from one lymphangion to another. While the rhythm was irregular, the sequence of contractions was consistent: 1, 2, 3, 4, 5, as shown by the positive values of inter-lymphangion delay; this sequence reflects the slight overall decrease in mean pressure along the chain. The extent of the delay showed an underlying consistency; although each delay varied from cycle to cycle, that from lymphangion 1 to lymphangion 2 was longest on average, while that from lymphangion 2 to lymphangion 3 was shortest on average. Only the outermost valves 1 and 6 operated in every cycle; valves 3, 4 and 5 operated approximately every alternate cycle, giving rise to a profile of mean pressures which shows that almost the entirety of the burden of resisting the adverse pressure difference across the chain was borne by the outermost valves. That the irregularity of cycles was chaotic, not merely repetitive over a long period, is proved by the recurrence plot.

¹The relatively small number of cycles computed here does not permit formal verification of dynamical chaos, but the recurrence plot clearly shows that while $\bar{Q}$ varies irregularly by a factor of 5.5, the system is following a trajectory which limits the returns to an attractor—they are not uniformly distributed. This is strongly indicative of chaos.

With all valves operating, albeit not in every cycle, the mechanical efficiency fell back to 47.7 %, a value slightly better than that exhibited with $t_p = 0.3$ s, but considerably lower than that with $t_p = 0$. However, the overall mean flow-rate, 45.7 $\mu\text{L/hr}$, was even lower than that for $t_p = 0$, and only 58 % of that for $t_p = 0.3$ s.

Despite the lack of a formal method of coordination when t_p was large, the lymphangions all adopted the same diastolic duration on average, and in consequence the chain of lymphangions maintained a fixed contraction sequence. However, this was not the case when the pressure difference faced by the chain was favourable. With $p_a = 6$ cmH₂O and $p_b = 3$ cmH₂O, the mean pressure declined along the chain, and each lymphangion established a different repetition rate (not shown).

3.3 Model outcomes – steps of both p_{inlet} and p_{outlet}

Using the model with what we regard as the physiological value of t_p (0.3 s), we also emulated both the step procedures applied to the isolated lymphatic segment, and others. Figures 9 and 10 show the results of applying elevated pressure to both the inlet and the outlet pressures (p_a and p_b) simultaneously. The two increases in pressure cause increases in the repetition rate of contractions, most easily seen in the traces of events for valves V5 and V6, which operated throughout every contraction/relaxation cycle. Other valves were not so persistent; V1 missed one closure at the time of the first step up, and valves V2 and V3 missed several closing opportunities in the baseline state and during the first pressure step. Valves V2 and V4 stayed open throughout the second step; thus during this time there were effectively only three pump chambers.

The intra-lymphangion pressures all increase with the boundary pressures, but there is a noticeably long adjustment transient after the first step starts. The lymphangion diameters also increase, but both mean level and the extent of diameter change are clearly restricted by the steepness of the passive (diastolic) $p_{\text{lm}}-D$ relationship at high pressures.

Further quantities from this simulation are plotted in Figure 10. The duration of diastole reduces as the boundary pressures increase, being a measure of the increase in contraction repetition rate, but non-linearly, reflecting the non-linear measured dependence of contraction frequency on p_{lm} [see fig. 2 of Bertram et al. (2017)]. The duration is noticeably irregular when $p_a = p_b = 3$ cm H₂O. The irregularity is best seen in the inter-lymphangion delays for L1 to L2, and L2 to L3, which in the baseline state range between +0.3 s and small negative values. After the first step up, to $p_a = p_b = 6$ cmH₂O at 60 s, this pair of delays modulates slowly, finally ranging only between 0.275 and 0.3 s after 85 s. At $p_a = p_b = 9$ cmH₂O, all delays are at the positive limit of $t_p = 0.3$ s. Positive values of delay mean that the contraction sequence is 1, 2, 3, 4, 5, unlike that found with the same value of t_p when p_b exceeded p_a by 4 cmH₂O (Figs. 5–7).

Reflecting the inter-lymphangion delays, the cycle-mean flow-rates are irregular in the baseline state, and go through an extended transient at $p_a = p_b = 6$ cmH₂O before settling into a period-2 alternation. The mean flow-rate at $p_a = p_b = 6$ cmH₂O is higher than at $p_a = p_b = 3$ cmH₂O, because the increased p_{lm} allows greater lymphangion filling in diastole,

but falls to an intermediate value at $p_a = p_b = 9 \text{ cmH}_2\text{O}$ where systolic circumferential shortening is reduced [see fig. 1B of Bertram et al. (2017)].

The pattern of intra-lymphangion mean pressures in the last cycle (Fig. 10, upper left) reflects what must happen when $p_a = p_b$. Increases in mean pressure across operating valves (V1, V3, V6—refer to Fig. 9) are balanced by decreases across valves which fail to close in that cycle (V2) or close only very briefly (V4, V5), and by viscous pressure drops for flow through all lymphangions and the inlet and outlet resistors.

3.4 Model outcomes – steps of p_{outlet} only

The procedure here is as in section 3.3, but now applied to p_b only, with p_a remaining at its initial value. After starting with equal boundary pressures, the lymphangion chain then faces two increases in adverse pressure difference across the pump; see Figures 11 and 12. As can be seen from the pressure and diameter traces, each step, including the return to baseline, gives rise to a prolonged adjustment transient; that following the first step takes more than 30 s and ten beats to settle, and even after that the mean flow-rate continues to change slightly in response to continuing variations in diastolic duration and the contraction delay between L4 and L5. Each increase in outlet pressure causes a reduction in the post-transient cycle-mean flow-rate, but thanks to the concomitant rate increases (reductions in diastolic duration), the flow-rate reductions are less than would have occurred otherwise. Nevertheless, the mean flow-rate when $p_b = 9 \text{ cmH}_2\text{O}$ is only half that when p_a was also 9 cmH₂O (Fig. 10). As when p_b was 7 cmH₂O (Figs. 5–7), the post-transient contraction sequence at $p_b = 9 \text{ cmH}_2\text{O}$ indicates retrograde conduction, with the last (part-)lymphangion controlling the rate for all lymphangions. However, the valve behaviour in response to setting $p_b = 6 \text{ cmH}_2\text{O}$ is more similar to that for $p_b = 7 \text{ cmH}_2\text{O}$ (cf. Figs. 11 and 5), with V5 staying open in both cases; the difference at $p_b = 6 \text{ cmH}_2\text{O}$ is that V2 opens and closes twice per cycle. It continues to do this at $p_b = 9 \text{ cmH}_2\text{O}$, but the valve that stays open is V4, not V5.

3.5 Model outcomes – steps of p_{inlet} only

As noted in the context of the animal experiment (Fig. 1), raising p_{inlet} above p_{outlet} means that the flow-rate through the lymphatic is no longer controlled by the extent of lymphangion pumping. The vessel acts more as a passive conduit, and continued contractions may even increase the resistance that the vessel offers to through flow. However, the current model emulates the behaviour found in Fig. 1, whereby contractions persisted at high flow-rates, in fact with increased repetition rate because of the raised mean systolic pressure, as shown in Figures 13 and 14. Although in this respect the model procedure is similar, and many similarities between the animal experiment outcomes and the model outcomes can be noted, the model outcomes are far from qualitatively identical. Thus in the real vessel, all three valves stayed open once p_{inlet} exceeded p_{outlet} , whereas in the model vessel, the last valve (V6) continued to operate cyclically throughout, and only when p_a reached 9 cmH₂O did valves V1–3 and V5 stay open continuously. The difference may relate to flow-rate magnitude; in the isolated segment, the measured flow-rates when $p_{\text{inlet}} > p_{\text{outlet}}$ exceeded that when $p_{\text{inlet}} = p_{\text{outlet}}$ many-fold (see Fig. 1), whereas in the model, the two steps of p_a only doubled then tripled the initial mean flow-rate. Nevertheless, already at

$p_a = 6 \text{ cmH}_2\text{O}$, the mean flow-rate of $200 \text{ }\mu\text{L/hr}$ exceeded that for any other procedure investigated.

Before, during and after the p_a steps, the model, like the real lymphatic segment, propagated contraction signals in the antegrade direction, giving rise to the contraction sequence 1, 2, 3, 4, 5. Once p_a was elevated above p_b , the delay between the contractions of adjacent lymphangions became fixed at approximately 0.3 s . A small adjustment in these intervals just before 150 s was associated with a non-negligible decrease in the diastolic duration in all lymphangions. When $p_a = 6 \text{ cmH}_2\text{O}$, valve V4 stayed open, and V2 closed only in alternate cycles. The intra-lymphangion pressures decreased along the chain, more so when p_a was elevated. Although the minimum intra-lymphangion pressure increased by more than $2 \text{ cmH}_2\text{O}$ when p_a reached $9 \text{ cmH}_2\text{O}$, the minimum diameter increased only slightly relative to the extent of diameter change during contraction; again this is similar to what was observed in the real segment (Fig. 1).

3.6 Comparison with a fixed-rate model

In response to steps of p_b alone, and of p_a and p_b together, the rate-responsive model varied its mean flow-rate less than a corresponding model in which the rate remained fixed (results not shown). The mean flow-rates and diastolic durations (post-transient where possible) during all combinations of p_a and p_b reached during the three step procedures are shown in Table 1. Also shown in the table (in bold) are values of mean flow-rate from a model in which diastolic duration was not a function of pressure. Instead, at each combination of p_a and p_b , the length of the relaxation period between contractions was set to a value approximating that arrived at by pressure feedback in the rate-responsive model. In every case, the mean flow-rates from the two models agree to within the level of accuracy of the comparison, showing that it was the changes in diastolic duration from the rate-responsive model which were solely responsible for the improved maintenance of mean flow-rate in the face of adverse pressure differences and increased transmural pressure.

3.7 Further model behaviour

The model is capable of exhibiting a range of contraction coordination behaviours besides those illustrated above; some of these are briefly described in this section. If the lymphangion chain is exposed to a range of outlet pressures, starting from $p_b = p_a$ and increasing p_b until eventually forward mean flow-rate ceases, it transitions through a range of coordination states, starting from the sequence 1, 2, 3, 4, 5 and progressing to 5, 4, 3, 2, 1 through various intermediate states. For instance, when $p_b = 5 \text{ cmH}_2\text{O}$, with all other parameters set as for Fig. 5, lymphangion 3 is the last to contract; contractions are independently initiated at lymphangions 1 and 5 (actually, at the part-lymphangions beyond), with L2 slaved to L1 (i.e. contracting t_p later) and L3 and L4 slaved to L5. Activation signals are propagated to L3 from L4, consistently beating those which would otherwise arrive $\sim 0.13 \text{ s}$ later from L2. The resulting sequence 5, 1, 4, 2, 3 is accompanied by valves V2 and V3 staying open continuously, and V4 cycling between open and closed states twice per contraction cycle.

When p_b is increased from 5 to 6 cmH₂O, the above contraction sequence inverts. Instead of contractions working their way inward along the chain, now the first lymphangion to contract is L4, with L5 following independently only ~0.05 s behind, and L3, L2 and L1 all slaved to L4. The resulting sequence 4, 5, 3, 2, 1 is accompanied by normal valve operation everywhere except at V5, which stays open continuously, converting L4 and L5 into effectively a single pump chamber. This behaviour involves the signals conducted from L4 to L5, and from L5 to L4, both being extinguished by arrival within each other's refractory periods.

Further increase of p_b to 7 cmH₂O brings the behaviour shown in Fig. 5, still involving V5 staying open continuously but now with all lymphangions slaved to L5.

4 Discussion

4.1 Animal model

Because of the commercial availability of many different genetic variants of the mouse, the latter has increasingly become the default animal model for lymphatic studies. However, our previous models, many parts of which serve as the foundation for this study, are all based on experimental data from the rat mesentery (e.g. valve opening behaviour, valve resistance, length-tension relationship). Although peripheral vessels from the mouse mostly behave similarly to those from rat mesentery, the mouse mesentery is unique among other species studied (rat, guinea pig, dog, sheep, pig, cow) in not having robust intrinsic contractions capable of propelling lymph (Zawieja et al. 2017; Zawieja et al. 2018). Human mesenteric lymph vessels mostly show vigorous spontaneous contractions (Telinius et al. 2017; Mohanakumar et al. 2018), as we have also observed in as-yet unpublished work.

4.2 Broad findings

Overall, the major conclusion of this paper is that a great variety and complexity of lymphangion coordination sequences can result merely from the introduction into a multi-lymphangion model of contraction-rate dependence on pressure. Since every lymphangion is qualitatively functionally similar, that in turn demands consideration of coordination between lymphangions. Otherwise each lymphangion would contract entirely independently of its neighbours up- and downstream, which is clearly contrary to the great majority of experimental observations. Our favoured mechanism for this coordination is the (electrical) conduction of activation signals between lymphangions, for which there is much evidence (Scallan et al. 2016). With pressure-dependent rate and conducted activation, rhythms found in the model include irregular ones (Fig. 10 and 14). Both the two-way outward propagation of activation signals originating from an intermediate lymphangion, and the extinguishing within the chain of colliding inwardly propagating signals, have been found. Both our experimental observation of antegrade propagation of contractions when $p_{\text{inlet}} < p_{\text{outlet}}$, and the change to retrograde propagation when $p_{\text{outlet}} > p_{\text{inlet}}$, have been explained. *In vivo*, a further complication is that activation signals do not always get conducted to adjacent lymphangions, or if they arrive do not always get acted upon (Zawieja et al. 1993). In addition, some pairs of adjacent lymphangions do not exchange conduction signals at all (Crowe et al. 1997). These variables, which were only emulated in the model by abolishing

conduction altogether, would provide still further rhythms and opportunities for irregularity, as would converging signals from branches. Our contention is thus that a hitherto unrealized large part of real (observed) lymphatic rhythm and coordination can be explained on the basis of pressure-dependent rate and conducted activation alone, without involving further regulatory mechanisms.

4.3 Model/experiment agreement

While the model successfully explains the major findings from the experiment, there are significant discrepancies between what was observed in the isolated segment and the outcomes of the model at a secondary level. These discrepancies have been detailed in section 3.5. It is important to realize that agreement at detail level is not to be expected. For one thing, there is a vast degree of biological variation, such that quite different secondary behaviour might be seen from a further experiment. However, this does not weaken the importance of what was found and illustrated in Fig. 4; the outcome of that one experiment shows clearly that manipulation of end-pressures can control the direction of propagation of the signals which coordinate lymphangion contractions. Secondly, the model is not quantitatively matched in detail to any one isolated segment, but incorporates properties of valve mechanics, passive lymphatic wall stiffness and active contraction which have been accumulated over years of separate and differing types of experiment, as reported in various papers (Davis et al. 2011; Bertram et al. 2014b, 2017). Consequently, while behaviour may differ in secondary ways when the model is compared with the experiment of Fig. 4, this does not mean that the model is not capable of reproducing such behaviour. For instance, it was noted in the context of Fig. 13 that whereas in the vessel segment, all valves stayed open once p_{inlet} exceeded p_{outlet} , at least one valve continued to operate when $p_a > p_b$ in the model. However, under conditions of reduced R_{Vn} involving $p_a > p_b$ (not shown), all valves do stay open.

4.4 Advantages of modelling

The great strength of the model is that it allows (numerical) experiments which would be impossible *in vivo* or *ex vivo*. In a model, all variables are under control; where no two biological experiments show the same outcome, for reasons which remain obscure and are simply attributed to ‘biological variation’, results from a computer model are fully reproducible. Not only is this true at the level of the vessel segment, but the model can also consist of precisely identical lymphangions, something which can never be guaranteed for a biological segment. For example we recently showed experimental evidence of quantitatively differing properties for valves located at opposite ends of the same lymphangion (Bertram et al. 2017), and such situations have been modelled by us. The ability to experiment on a numerical segment consisting of lymphangions with identical properties immediately removes a major source of uncertainty in interpreting the results of corresponding biological experiments. Furthermore, parameter values can be varied at will numerically, where they are usually inaccessible experimentally. For instance, here we chose to investigate $t_p = 0$ and $t_p \rightarrow \infty$, both of which illuminated further the reasons for, and contrasted with, the model’s behaviour at more realistic values of this parameter. *Ex vivo*, the best one might do is to inhibit inter-lymphangion conduction pharmacologically, but this would both be irreversible, at least in the short term, and run the risk of changing other

aspects of vessel contractile behaviour, since inter-lymphangion conduction is thought to occur via muscle cells (Crowe et al. 1997; Scallan et al. 2016).

4.5 Other models of lymphangion coordination

More elaborate models of lymphangion coordination are the subject of current work. Baish et al. (2016) define a single-lymphangion model consisting of complementary oscillators describing mechanical stretch with calcium ion (Ca^{2+}) transport and fluid shear stress-induced nitric oxide (NO) production. They showed that the model synchronized to external stimuli, demonstrating in principle that it provides a means of coordinating lymphangions, although they did not explore this. In an earlier paper, the same group showed that a version of this model predicted retrograde propagation of contraction waves when the shear-induced NO dynamics model was switched on, and antegrade propagation when it was switched off, leaving just the Ca^{2+} oscillator (Kunert et al. 2015; Kunert et al. 2016). Another model based on stretch-activated calcium influx and contraction inhibition by wall shear stress has been proposed by Contarino & Toro (2017), although full details have yet to be published. No specific coupling between adjacent lymphangions was included; lymphangions exhibit the same phase and contraction frequency if started with identical initial conditions, but desynchronize when subjected to different pressures.

Our model is purely phenomenological; it does not attempt to emulate at a cellular level the physiological processes underlying contraction rate increase with pressure, inter-lymphangion signalling, and the development of a refractory state to such signalling during and shortly after contraction. As such it retains maximum simplicity commensurate with fidelity to the corresponding phenomena observed biologically.

4.6 Dependence of rate on wall shear

An inverse dependence of contraction rate on wall shear was also included in the model of Caulk et al. (2016), although this has not been extended beyond a single pumping lymphangion. Their model has contraction frequency initially set via a nonlinear dependence on the average of the prescribed vessel inlet and outlet pressures, with the frequency/pressure relationship based on data from Davis et al. (2012). Subsequently this frequency is modified through a comparison of time-averaged shear with a set value, with the modification governed by a 5-minute time constant. The frequency/shear relation was based on the data of Gashev et al. (2002), which show progressive reduction in normalized contraction frequency with increase in $p_{\text{inlet}} - p_{\text{outlet}}$ in isolated lymphatic segments, keeping $p_{\text{inlet}} + p_{\text{outlet}}$ constant. Recently the same group have themselves contributed to the literature on contraction rate vs. shear, using apparatus which enabled estimation of 5s-averaged wall shear stress from the imposed flow-rate, continuously measured vessel diameter, and assumed fluid viscosity and velocity profile (Kornuta et al. 2015).

Here we have chosen not to model shear-induced frequency reduction. Rather we have analysed the coordination of multiple lymphangions when each is able to vary its contraction frequency independently based on its local pressure in the immediately preceding contraction. Even with this change alone, the ensuing analysis has revealed many hidden

complexities. In principle the analysis could be readily extended to include frequency/shear dependence. We will be reporting in due course on such a model.

4.7 Chaos at large propagation time

From a dynamical systems standpoint, it is of interest that the multi-lymphangion model with locally determined pressure-dependent relaxation time exhibits dynamical chaos. It seems that chaotic or pre-chaotic² behaviour can be manifested whenever the pressure boundary conditions are such that one lymphangion does not impose its rate on all the rest via inter-lymphangion signalling, or equivalently when such signalling is abolished by making the value of t_p large. While the system certainly fulfils the basic criteria for chaotic response in terms of nonlinearity and number of degrees of freedom, this was still an unexpected outcome of imposing a tightly organized scheme of rate variation.

4.8 Transients and their persistence

The simulations also revealed a great variety of duration of transients, both those engendered by start-up conditions and those following a step change of boundary pressure conditions. In general a chain of n lymphangions can be expected to take at least n cycles of contraction for the effects of start-up conditions to decay, and typically rather longer. Thus in the case of the simulation shown in Figs. 5–7, although repetitive conditions were largely reached after some 20–25 s, a completely steady state was not reached until ~40 s. Longer transients resulted when there was prolonged variation of inter-lymphangion delays within the $\pm t_p$ limits. Thus as seen in Fig. 12, a transient of >60 s followed³ the stepping of p_b from $p_b = p_a = 3$ cmH₂O to 6 cmH₂O. Other simulations (not shown here) have given rise to post-start meandering⁴ of the system's operating point with no apparent end; for instance, with $p_a = 3$ and $p_b = 14$ cmH₂O, the integration was continued for 150 s of simulated time (some 60 contraction cycles) without finding a settled state (between 110 and 122 s it appeared to find a steady cycle-mean flow-rate shared by all lymphangions, before the outbreak of another large operating-point migration).

4.9 Antegrade and retrograde propagation

The present model relies on the idea that any lymphangion can take on the role of pacemaker when its local conditions of pressure so dictate. Pacemaker here refers to an individual lymphangion within a chain or network of other lymphatic vessel segments. In the literature the term can also refer to the individual cell (or group of cells) in the lymphangion that is (are) initiating action potentials to drive contractions of the entire lymphangion, and perhaps adjacent lymphangions. We do not address this second usage here.

As indicated in section 1, the propagation that is incorporated here is likely to be electrically conducted between lymphatic muscle cells. There is also some evidence for mechanical

²An example of pre-chaotic behaviour occurred with $p_a = 3$, $p_b = 4$ cmH₂O; inter-lymphangion delays 3 to 4, and 4 to 5, were locked to $+t_p$, while delay 1 to 2 varied between 0.09 s and t_p , and delay 2 to 3 between -0.11 and $+0.23$ s, in a stable period-5 rhythm which was also seen in all the cycle-mean flow-rates and diastolic durations. Similarly, a post-transient period-3 rhythm associated with V2 staying open in one of every three contraction cycles occurred at $p_a = p_b = 6$ cmH₂O (see Figs. 9 and 10), and period-2 rhythm resulted from V2 staying open in alternate cycles at $p_a = 6$, $p_b = 3$ cmH₂O (see Figs. 13 and 14).

³Only slightly greater than 60 s, as was proved in a separate run (not shown).

⁴This term is used to distinguish these behaviours from the steady chaos exhibited in Fig. 8.

coupling playing a role in coordinating contraction between lymphangions (Crowe et al. 1997), but this is not likely to be the major element. Inter-lymphangion conduction by mechanical means would be extremely asymmetrical because of the valves (not just slightly as in our experiment), and would create longer time lags than those we measured. Also, in as-yet unpublished work, one of us (MJD) has observed conduction proceeding between partial lymphangions in the (almost complete) absence of the transmission of a mechanical pressure pulse.

The numerical results here suggest a reason why retrograde conduction is so prevalent *in vivo*, manifesting as often as antegrade; Zawieja et al. (1993) found in 13 experiments that between 15 and 100 % of contractions were conducted retrograde to lymph flow, with mean 46.2 %. The mean-pressure profile for collecting lymphatics when pumping has pressure rising in the direction of lymph flow (Aukland and Reed 1993), because successive lymphangions step up the mean pressure until the high hydraulic resistance of a lymph node (Browse et al. 1984) is encountered. Under the rate scheme modelled here, this would naturally lead to the last lymphangion controlling rate for those in the vessel upstream. Conversely, under conditions where upstream and downstream pressures do not differ greatly, both our experiment and our simulations show that antegrade propagation is to be expected. Together, these two conditions may explain the observed prevalence of both antegrade and retrograde propagation, although the experiments of Zawieja et al. (1993) did not include monitoring of pressure.

5 Conclusions

It is universally observed in isolated metabolically supported segments that the rate of lymphatic contractions increases with distending pressure. To include such dependence in our multiple-lymphangion model of the pumping by collecting lymphatics, it is essential to consider how the contractions of individual lymphangions are coordinated in time, as observed *in vivo*. Since every lymphangion is capable of pressure-regulated pace-making, and it was found by experiment that the order of contractions could be reversed by manipulation of the pressure gradient, we adduced principles for a scheme of conducted activation which is consistent with known physiology. We implemented the scheme in our model, where it reproduced our finding of pressure-governed conduction reversal, and yielded coordinated contractions. Exploring the behaviour of the resulting model further, we found that the precise detail of how the reversal was effected across several lymphangions varied with the external conditions. Furthermore, under some conditions the post-starting-transient pumping state did not arrive at a steady rate of contractions, but varied in complex ways (period-3, etc.), including chaotic rhythms.

We believe that this phenomenological model of pressure-controlled rate in multiple lymphangions is the simplest implementation that is consistent with what is known of the relevant lymphatic behaviour as revealed by experiment over decades. To our knowledge, this is the first time that a computer model of a lymphatic vessel has specifically organized the coordination of several lymphangions in a way based on physiological principles, although aspects of the issue have been addressed at the level of intracellular behaviour and resulting dynamics by Baish et al. (2016). The unexpectedly wide range of behaviour

manifested by the model suggests that some instances of irregular contraction seen *in vivo* may be the result of similar simple governing principles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix 1: equations for n lymphangions with inlet/outlet pipette resistance

Mass conservation

$$\frac{dD_i}{dt} = \frac{D_i^3}{32\mu L^2} (p_{1,i} - 2p_{m,i} + p_{2,i}), \text{ for } i=1 \text{ to } n; D = \text{diameter, } t = \text{time, } \mu = \text{viscosity, } L =$$

lymphangion length, p = pressure, and suffices 1, m and 2 denote the upstream end, mid-point, and downstream end of a lymphangion⁵.

Momentum conservation

$$p_{1,i} - p_{m,i} = \frac{64\mu L}{\pi D_i^4} R_V \frac{p_{2,i-1} - p_{1,i}}{R_V(p_{2,i-1}, p_{1,i}; p_e)},$$

$$\text{and } p_{m,i} - p_{2,i} = \frac{64\mu L}{\pi D_i^4} R_V \frac{p_{2,i} - p_{1,i+1}}{R_V(p_{2,i}, p_{1,i+1}; p_e)}, \text{ where}$$

$$R_{V,i} = R_{Vn} + \frac{R_{Vx}}{1 + \exp[-s_0(\Delta p_{V,i} - \Delta p_{o,i})]}, \text{ with } \Delta p_{V,i} = p_{2,i-1} - p_{1,i};$$

R_V = valve resistance, R_{Vn} = minimum valve resistance, $R_{Vx} + R_{Vn}$ = maximum valve resistance, and s_0 = a constant defining the rate of transition. The open/close threshold $p_{o,i}$ is a nonlinear function of $p_{2,i-1} - p_e$ when closed, and of $p_{1,i} - p_e$ when open; see Bertram et al. (2014b).

For $i=1$ only, $p_{2,i-1} = p_{2,0} = p_a - R_a \frac{p_{2,0} - p_{1,1}}{R_{V,1}}$; For $i=n$ only,

$$p_{1,i+1} = p_b + R_b \frac{p_{2,n} - p_{1,n+1}}{R_{V,n+1}}; p_a \text{ and } p_b \text{ are the pressures in the up- and downstream}$$

⁵Relative to our previous papers on this model, the suffix order has been changed; thus for instance what was previously p_{im} is now $p_{m,i}$.

reservoirs, and R_a and R_b are the resistances of the up- and downstream micropipettes cannulating the vessel segment, respectively.

Constitutive relations: the passive and maximally contracted states are defined by the curves $D_{\dot{p}_{psv}} = f_{psv}(p_{tm,i})$; and $D_{\dot{p}_{act}} = f_{act}(p_{tm,i})$ respectively, where $p_{tm,i} = p_{m,i} - p_e$; see Bertram et al. (2017). A contraction consists of smooth evolution of the $D_f(p_{tm,i})$ -relation between these extremes governed by $D_i = D_{\dot{p}_{psv}} - M(t)[D_{\dot{p}_{psv}} - D_{\dot{p}_{act}}]$, where the time-course of active contraction $M(t)$ is calculated as detailed by Bertram et al. (2017); at its simplest, it is an inverted cosine wave with frequency f and duration $1/f$.

Pressure-dependent diastolic duration: the period of relaxation between systoles (in the absence of activation signals conducted from other lymphangions) is calculated at each instant of end-systole as $\Delta t_{r,i} = \frac{1}{f_{tw,i}} - \frac{1}{f}$, where $f_{tw,i} = 60y_i$ with $y_i = -1.39q_i^2 + 12.6q_i$

+ 0.647, $q_i = \ln(1 + x_i)$, and $x_i = f \int_{t_{c,i}}^{t_{c,i} + 1/f} \Delta p_{tm,i} dt$ is the average transmural pressure (in cmH₂O) over the preceding systole starting at time t_c ; see Bertram et al. (2017)⁶.

Appendix 2: numerical scheme for organizing contraction times

Two vectors are maintained throughout the simulation, each of length equal to the number of lymphangions plus two (i.e. including the two part-lymphangions at the ends).

The first vector, say s_{old} , contains the times at which each lymphangion last began contraction. The second, say s_{new} , contains the most up-to-date information about the times at which each lymphangion will next start contraction. The algorithm is as follows:

1. Initialise: at $t = t_0$, all lymphangions start contracting, so set the vector elements $s_{old}(i) = t_0$ for all i . Set $s_{new}(i) = t_{large}$ for all i , where t_{large} is some large positive value greater than the maximum time in the full simulation.
2. At the conclusion of each (full) timestep dt in the Runge-Kutta integration, at time t :
 - a. For each lymphangion L_i if $s_{new}(i)$ lies in the interval $[t - dt, t]$, initiate contraction of lymphangion L_i update $s_{old}(i) = s_{new}(i)$, and propagate the signals to nearest-neighbour lymphangions L_{i-1} and L_{i+1} . These lymphangions may then initialise contraction after a delay t_p by updating $s_{new}(i+1) = \min(s_{new}(i+1), t + t_p)$ and similarly for $s_{new}(i-1)$, but the updates can only occur if they are outside the current relevant refractory period, e.g. for the case of the neighbouring lymphangion L_{i+1} , set $s_{new}(i+1) = t + t_p$ only if $t + t_p > s_{old}(i+1) + t_n$.
 - b. For each lymphangion L_i if the time of conclusion of a contraction lies in the interval $[t - dt, t]$, update $s_{new}(i) = \min(s_{old}(i) + t_n, t + t_r)$, where

⁶The definition of x_i here corrects an error in the equivalent Appendix eq. 8 of Bertram et al. (2017).

t_r is calculated according to the average of $p_{tm}(t)$ over the just-concluded contraction.

References

- Aukland K, Reed RK. Interstitial-lymphatic mechanisms in the control of extracellular fluid volume. *Physiol Rev.* 1993; 73(1):1–78. [PubMed: 8419962]
- Baish JW, Kunert C, Padera TP, Munn LL. Synchronization and random triggering of lymphatic vessel contractions. *PLoS Computational Biology.* 2016; 12(12):e1005231.doi: 10.1371/journal.pcbi.1005231 [PubMed: 27935958]
- Bertram CD, Macaskill C, Davis MJ, Moore JE jr. Development of a model of a multi-lymphangion lymphatic vessel incorporating realistic and measured parameter values. *Biomechanics and Modeling in Mechanobiology.* 2014b; 13(2):401–416. DOI: 10.1007/s10237-013-0505-0 [PubMed: 23801424]
- Bertram CD, Macaskill C, Davis MJ, Moore JE jr. Consequences of intravascular lymphatic valve properties: a study of contraction timing in a multi-lymphangion model. *American Journal of Physiology - Heart and Circulatory Physiology.* 2016b; 310(7):H847–H860. DOI: 10.1152/ajpheart.00669.2015 [PubMed: 26747501]
- Bertram CD, Macaskill C, Davis MJ, Moore JE jr. Valve-related modes of pump failure in collecting lymphatics: numerical and experimental investigation. *Biomechanics and Modeling in Mechanobiology.* 2017; 16(6):1987–2003. DOI: 10.1007/s10237-017-0933-3 [PubMed: 28699120]
- Bertram CD, Macaskill C, Moore JE jr. Incorporating measured valve properties into a numerical model of a lymphatic vessel. *Computer Methods in Biomechanics and Biomedical Engineering.* 2014a; 17(14):1519–1534. DOI: 10.1080/10255842.2012.753066 [PubMed: 23387996]
- Bertram CD, Macaskill C, Moore JE jr. Pump function curve shape for a model lymphatic vessel. *Med Eng Phys.* 2016a; 38(7):656–663. DOI: 10.1016/j.medengphy.2016.04.009 [PubMed: 27185045]
- Browse NL, Doig RL, Sizeland D. The resistance of a lymph node to lymph flow. *Br J Surg.* 1984; 71:192–196. [PubMed: 6697120]
- Caulk AW, Dixon JB, Gleason RL jr. A lumped parameter model of mechanically mediated acute and long-term adaptations of contractility and geometry in lymphatics for characterization of lymphedema. *Biomechanics and Modeling in Mechanobiology.* 2016; 15(6):1601–1618. DOI: 10.1007/s10237-016-0785-2 [PubMed: 27043026]
- Contarino C, Toro EF. Modelling the lymphatics: a 1D lymph flow model coupled to an electro-fluid-mechanical contraction model; Paper presented at the 5th International Conference on Computational and Mathematical Biomedical Engineering - CMBE2017; Pittsburgh, PA, USA. 10-12 April. 2017.
- Crowe MJ, von der Weid P-Y, Brock JA, Van Helden DF. Co-ordination of contractile activity in guinea-pig mesenteric lymphatics. *J Physiol.* 1997; 500(1):235–244. [PubMed: 9097947]
- Davis MJ. An improved, computer-based method to automatically track internal and external diameter of isolated microvessels. *Microcirculation.* 2005; 12(4):361–372. DOI: 10.1080/10739680590934772 [PubMed: 16020082]
- Davis MJ, Davis AM, Ku CW, Gashev AA. Myogenic constriction and dilation of isolated lymphatic vessels. *Am J Physiol (Heart Circ Physiol).* 2009a; 296(2):H293–H302. [PubMed: 19028793]
- Davis MJ, Davis AM, Lane MM, Ku CW, Gashev AA. Rate-sensitive contractile responses of lymphatic vessels to circumferential stretch. *J Physiol.* 2009b; 587(1):165–182. DOI: 10.1113/jphysiol.2008.162438 [PubMed: 19001046]
- Davis MJ, Rahbar E, Gashev AA, Zawieja DC, Moore JE jr. Determinants of valve gating in collecting lymphatic vessels from rat mesentery. *American Journal of Physiology - Heart and Circulatory Physiology.* 2011; 301(1):H48–H60. DOI: 10.1152/ajpheart.00133.2011 [PubMed: 21460194]
- Davis MJ, Scallan JP, Wolpers JH, Muthuchamy M, Gashev AA, Zawieja DC. Intrinsic increase in lymphangion muscle contractility in response to elevated afterload. *American Journal of Physiology - Heart and Circulatory Physiology.* 2012; 303(7):H795–H808. DOI: 10.1152/ajpheart.01097.2011 [PubMed: 22886407]

- Gashev AA, Davis MJ, Delp MD, Zawieja DC. Regional variations of contractile activity in isolated rat lymphatics. *Microcirculation*. 2004; 11(6):477–492. [PubMed: 15371129]
- Gashev AA, Davis MJ, Zawieja DC. Inhibition of the active lymph pump by flow in rat mesenteric lymphatics and thoracic duct. *J Physiol*. 2002; 540(Pt. 3):1023–1037. DOI: 10.1113/jphysiol.2002.016642 [PubMed: 11986387]
- Jamalian S, Davis MJ, Zawieja DC, Moore JE jr. Network scale modeling of lymph transport and its effective pumping parameters. *PLoS One*. 2016; 11(2):e0148384. (18 pages). doi: 10.1371/journal.pone.0148384 [PubMed: 26845031]
- Jamalian S, Jafarnejad M, Zawieja SD, Bertram CD, Gashev AA, Zawieja DC, Davis MJ, Moore JE jr. Demonstration and analysis of the suction effect for pumping lymph from tissue beds at subatmospheric pressure. *Scientific Reports*. 2017; 7:1–17. article 12080. DOI: 10.1038/s41598-017-11599-x [PubMed: 28127051]
- Kornuta JA, Nepiyushchikh ZV, Gasheva OY, Mukherjee A, Zawieja DC, Dixon JB. Effects of dynamic shear and transmural pressure on wall shear stress sensitivity in collecting lymphatic vessels. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*. 2015; 309(9):R1122–R1134. DOI: 10.1152/ajpregu.00342.2014
- Kunert C, Baish JW, Liao S, Padera TP, Munn LL. Mechanobiological oscillators control lymph flow. *Proceedings of the National Academy of Sciences of the USA*. 2015; 112(35):10938–10943. DOI: 10.1073/pnas.1508330112 [PubMed: 26283382]
- Kunert C, Baish JW, Liao S, Padera TP, Munn LL. Letter - Reply to Davis: Nitric oxide regulates lymphatic contractions. *PNAS*. 2016; 113(2):E106.doi: 10.1073/pnas.1522233113 [PubMed: 26719425]
- McGeown JG, McHale NG, Roddie IC, Thornbury K. Peripheral lymphatic responses to outflow pressure in anesthetized sheep. *J Physiol*. 1987; 383:527–536. [PubMed: 3656133]
- McHale NG, Meharg MK. Co-ordination of pumping in isolated bovine lymphatic vessels. *J Physiol*. 1992; 450:503–512. [PubMed: 1432715]
- McHale NG, Roddie IC. The effect of transmural pressure on pumping activity in isolated bovine lymphatic vessels. *J Physiol*. 1976; 261(2):255–269. [PubMed: 988184]
- Mohanakumar S, Majgaard J, Telinius N, Katballe N, Pahle E, Hjortdal VE, Boedtker DMB. Spontaneous and α -adrenoceptor-induced contractility in human collecting lymphatic vessels require chloride. *American Journal of Physiology - Heart and Circulatory Physiology*. 2018; (in press). doi: 10.1152/ajpheart.00551.2017
- Scallan JP, Zawieja SD, Castorena-Gonzalez JA, Davis MJ. Lymphatic pumping: mechanics, mechanisms and malfunction. *J Physiol*. 2016; 594(20):5749–5768. DOI: 10.1113/JP272088 [PubMed: 27219461]
- Telinus N, Majgaard J, Mohanakumar S, Pahle E, Nielsen J, Hjortdal V, Aalkjær C, Boedtker DB. Spontaneous and evoked contractility of human intestinal lymphatic vessels. *Lymphatic Research and Biology*. 2017; 15(1):17–22. DOI: 10.1089/lrb.2016.0039 [PubMed: 28277905]
- Zawieja DC. Contractile physiology of lymphatics. *Lymphatic Research and Biology*. 2009; 7(2):87–96. DOI: 10.1089/lrb.2009.0007 [PubMed: 19534632]
- Zawieja DC, Davis KL, Schuster R, Hinds WM, Granger HJ. Distribution, propagation, and coordination of contractile activity in lymphatics. *American Journal of Physiology - Heart and Circulatory Physiology*. 1993; 264(4, Pt. 2):H1283–H1291.
- Zawieja SD, Castorena-Gonzalez JA, Dixon JB, Davis MJ. Experimental models used to assess lymphatic contractile function. *Lymphatic Research and Biology*. 2017; 15(4):331–342. DOI: 10.1089/lrb.2017.0052 [PubMed: 29252142]
- Zawieja SD, Castorena-Gonzalez JA, Scallan JP, Davis MJ. Differences in L-type Ca^{2+} channel activity partially underlie the regional dichotomy in pumping behavior by murine peripheral and visceral lymphatic vessels. *American Journal of Physiology - Heart and Circulatory Physiology*. 2018; 314(5):H991–H1010. DOI: 10.1152/ajpheart.00499.2017 [PubMed: 29351458]

Figure 1.

Image shows the part of the vessel between valves 1 and 3 (flow from left to right); scale-bar = 100 μm . The traces below show the flow-rate, the state of each valve (1 = open, 0 = shut), the reservoir pressures, and the vessel diameter at the three sites indicated in the image.

Figure 2.

The three internal diameter traces relating to the steps of p_{out} are shown. Green lines indicate the detected times of contraction start.

Figure 3.

Waveforms of activation timing for a chain of six non-contracting pseudo-lymphangions (second panel), generated in response to a spatial and temporal variation of lymphangion pressures (top panel). Pseudo-lymphangion number increases along the chain in the direction of lymph flow; in the full pumping model, the part-lymphangions at the inlet (Lu) and outlet (Ld) ends will participate in the generation of activation signals but not in contractions. Each activation lasts 2 s; here $t_p = 0.25$ s and the refractory period $t_n = 2.5$ s. Relaxation time t_r is a nonlinear inverse function of local pressure. Third panel: diastolic duration, computed from the activation waveforms. Fourth panel: inter-lymphangion delay, computed from the activation waveforms.

Figure 4.

Result of applying steps of p_{outlet} (left panel) and p_{inlet} (right panel) to the isolated 2-lymphangion segment. Upper panels: the reservoir pressures p_{inlet} (blue) and p_{outlet} (red). Lower panels: the differences between the times of contraction start at site 2 relative to site 1 (blue), and at site 3 relative to site 2 (green). Positive (negative) values indicate that the start was later downstream (upstream). The occasional large 'spikes' probably represent failures of the detection algorithm.

Figure 5.

Upper left panel: pressures — p_a and p_b are the fixed boundary pressures (values as in the panel title), p_{02} is the pressure just before valve 1, p_{m} is the pressure at the mid-point of lymphangion i , for $i = 1$ to 5, and p_{p1} is the pressure just after valve 6. The pressure did not vary substantially along the length of a given lymphangion, so p_{i1} , p_{m} and p_{i2} followed essentially the same path. Lower left panel: the diameters of the five lymphangions. Upper right panel: waveforms of contraction activation. Lower right panel: the open/closed state of the six valves.

Figure 6.

Mean pressures p_{1i} , p_{mi} and p_{2i} in each lymphangion, over the last of a number of identical cycles (56.9 to 60 s). The locations of inter-lymphangion valves are indicated by vertical lines. Viscous dissipation causes a decline of pressure in the flow direction everywhere except across a valve that is operating to prevent backflow.

Figure 7.

From the top, the duration of diastole in each cycle, the delay between onset of contractions in adjacent lymphangions, and the cycle-mean flow-rate through each valve. Conditions as for Fig. 5, but here the run is continued out to 60 s.

Figure 8.

Left-hand panels, from top: diastolic duration, inter-lymphangion delay, cycle-mean flow-rate, and valve state, over 120 s of simulated time, with $t_p = 100$ s and all other parameters unchanged. Right-hand panels, from top: mean pressures in all lymphangions over the cycle ending at 120 s, and a first-return recurrence plot of $\bar{Q}_6(i)$ vs $\bar{Q}_6(i+1)$ i.e. the mean flow-rate through valve V6 in each cycle vs. that same mean flow-rate in the next cycle, for the same run but continued out to 600 s.

Figure 9.

Results of stepping both p_a and p_b from 3 to 6 to 9 and back to 3 cmH₂O, with the changes occurring at 60, 120, and 180 s. Top panel: diameter of each lymphangion. Middle panel: pressures $p_a = p_b$, p_{02} , p_{im} for $i = 1$ to 5, and p_{p1} (see caption to Fig. 5 for further explanation). Bottom panel: the state of each of the six valves. In contrast to the run shown in Figs. 5–7, here the start-up transient is complete within two contraction cycles.

Figure 10.

Left panels: mean pressures in each lymphangion during the final 'cycle' of the procedure shown in Fig. 9, and cycle-mean flow-rate through each valve in every cycle. Right panels: inter-lymphangion delays and diastolic durations.

Figure 11.

Diameters, pressures and valve states in response to stepping p_b from 3 to 6 to 9 and back to 3 cmH₂O, with the steps occurring at 60, 120, and 180 s. As Fig. 9 before the first step, so first 50s not shown here. See caption to Fig. 9 for further explanation of individual panels.

Figure 12.

Diastolic duration for each lymphangion, inter-lymphangion delays, and cycle-mean flow-rate through each valve, for the p_b -step procedure shown in Fig. 11.

Figure 13.

Diameters, pressures and valve states in response to stepping p_a from 3 to 6 to 9 and back to 3 cmH₂O, with the steps occurring at 60, 120, and 180 s. As Fig. 9 before the first step, so first 50s not shown here. See caption to Fig. 9 for further explanation of individual panels.

Figure 14.

Diastolic duration for each lymphangion, inter-lymphangion delays, and cycle-mean flow-rate through each valve, for the p_a -step procedure shown in Fig. 13.

Table 1

Comparison of mean flow-rate $\bar{Q}$ (ml/hr) between the model with $t_r = f(p_{lm})$ and (bold) one with fixed t_r (s), the value of which is adjusted to match approximately that arrived at in the rate-responsive model. The value of inter-lymphangion delay in the fixed- t_r model was set to $t_d = +0.3$ s, enforcing the contraction sequence 1, 2, 3, 4, 5.

	$p_b = 3 \text{ cmH}_2\text{O}$	$p_b = 6 \text{ cmH}_2\text{O}$	$p_b = 9 \text{ cmH}_2\text{O}$
$p_a = 3 \text{ cmH}_2\text{O}$	$t_r \approx 2.35, \bar{Q} \approx 0.09$ $t_r = \mathbf{2.35}, \bar{Q} = \mathbf{0.091}$	$t_r \approx 1.3, \bar{Q} \approx 0.08$ $t_r = \mathbf{1.3}, \bar{Q} = \mathbf{0.077}$	$t_r \approx 0.825, \bar{Q} \approx 0.06$ $t_r = \mathbf{0.825}, \bar{Q} = \mathbf{0.057}$
$p_a = 6 \text{ cmH}_2\text{O}$	$t_r \approx 1.4, \bar{Q} \approx 0.20$ $t_r = \mathbf{1.4}, \bar{Q} = \mathbf{0.199}$	$t_r \approx 1.15, \bar{Q} \approx 0.145$ $t_r = \mathbf{1.15}, \bar{Q} = \mathbf{0.141}$	
$p_a = 9 \text{ cmH}_2\text{O}$	$t_r \approx 0.9, \bar{Q} \approx 0.315$ $t_r = \mathbf{0.9}, \bar{Q} = \mathbf{0.311}$		$t_r \approx 0.8, \bar{Q} \approx 0.115$ $t_r = \mathbf{0.8}, \bar{Q} = \mathbf{0.115}$