Boundary waves in a microfluidic device as a model for intramural periarterial drainage

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ABSTRACT

The failure to clear amyloid-Beta from an aging brain leads to its accumulation within the walls of arteries and potentially to Alzheimer's disease. However, the clearance mechanism through the intramural periarterial pathway is not well understood. We previously proposed a hydrodynamic reverse transport model for the cerebral arterial basement membrane pathway. In our model, solute transport results from fluidic forcing driven by the superposition of forward and reverse propagating boundary waves. The aim of this study is to experimentally validate this hydrodynamic reverse transport mechanism in a microfluidic device where reverse transport in a rectangular conduit is driven by applying waveforms along its boundaries. Our results support our theory that while the superimposed boundary waves propagate in the forward direction, a reverse flow in the rectangular conduit can be induced by boundary wave reflections. We quantified the fluid transport velocity and direction under various boundary conditions and analyzed numerical simulations that support our experimental findings. We identified a set of boundary wave parameters that achieved reverse transport, which could be responsible for intramural periarterial drainage of cerebral metabolic waste.

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I. INTRODUCTION

Alzheimer's disease (AD) is a degenerative brain disease common in the elderly.¹ A characteristic of AD is the accumulation of a protein fragment, amyloid-Beta ($A\beta$), in the brain.^{2,3} Its accumulation is determined by the rate of $A\beta$ generation versus clearance.⁴ It is widely believed that age-related degradation of the normal clearance mechanisms contributes to AD.^{4,5,6} This evidence comes from the observations from human post-mortem studies,^{7,8} development of transgenic mouse models,⁹ and experimental studies that track fluorescent soluble tracers within 5 min of injection.¹⁰ $A\beta$ clearance occurs through proteolytic degradation,⁴ receptor-mediated transport,⁴ and possibly also flow along intramural periarterial structures.^{6,10} There is disagreement on how interstitial fluid (ISF), carrying $A\beta$ with it, might reach the lymph system.¹¹ The mechanisms are not well understood, with some advocating for perivenous transport and others for intramural periarterial migration. One objection to the perivenous pathway is the accumulation of $A\beta$ in the intramural periarterial spaces, known as cerebral amyloid angiopathy (CAA), which is very prevalent in AD. An objection to the intramural periarterial drainage (IPAD) is the

lack of a known mechanism that could pump ISF in an annular pathway surrounding an artery in a direction opposite to the blood flow direction. This periarterial space is not an empty chamber but is filled with smooth muscle cells. It is also layered with several basement membranes and is enclosed on the outside by the astrocyte feet. Our work aims to explore the conditions under which reverse transport in an annular region surrounding an elastic tube might be achieved. We adopt a greatly simplified geometric model that, while abstracting away much anatomical detail, concentrates on the boundary wave conditions needed for reverse transport. Thus, we make no precise mapping to the details of the intramural periarterial anatomy.

Several transport theories have been proposed to account for IPAD (Fig. 1). The first is the presence of global pressure gradients in the brain.¹² However, this theory would not explain the preferential ISF IPAD over the perivenous pathways. A second theory is that drainage is achieved by $A\beta$ selectively attaching to and detaching from the arterial walls during each pulse cycle to yield a net direction.¹² This theory is challenged by the experimental observation that fluorescent tracers without specific binding tendencies

FIG. 1. Schematic showing IPAD of the ISF in the brain. Direction of the blood flow is assigned to be forward, while the ISF flows co-axially in the reverse direction (opposite to blood flow) in the intramural periarterial region. The IPAD of the ISF flows between the layers of smooth muscle cells.

also travel in the reverse direction through the perivascular channels.¹⁰ Another theory suggests that within the intramural periarterial region, there could exist some flexible structures whose orientations present a greater flow resistance in the forward direction than in the reverse direction.^{13,14}

We have previously proposed a hydrodynamic reverse transport theory for IPAD driven by forward and reverse propagating boundary waves.¹⁵ We view this theory as a favorable candidate because the forward propagating waves are directly linked to the heartbeat, while the reverse waves could potentially be generated by wave reflections, astrocyte coordination, or vasomotor pulsation.^{16,17} In the case of wave reflections, these are a physiological phenomenon occurring at arterial branching junctions or any other

sites of sudden change in arterial geometry and/or elastic properties.^{18,19} For astrocyte coordination, given their known long-range communication mechanism using Ca^{2+} ion waves,^{20,21} it is conceivable that they could produce a reverse deformation wave through a long-range coordination of their end feet actions in constricting and expanding cerebrovascular surfaces.²¹ Note that regardless of their origin, our theory simply requires the presence of forward and reverse propagating waves.

The literature reports divergent results in supporting different IPAD theories.²² For example, there is evidence that A β clearance occurs along the periarterial basement membranes of capillaries and arteries,¹⁰ and along the parivascular spaces of capillaries, arteries, and veins.^{6,11} A major difference in these studies is the flow direction of ISF relative to blood flow. Despite these differences, these studies suggest that heart pulsations contribute to A β clearance out of the brain. It may be possible that either pulse waves or synchronized squeezing waves by the perivascular smooth muscle cells lead to the reverse IPAD observed by Carare *et al.*,¹⁰ while other mechanisms drive cerebrospinal fluid to flow in the direction of blood flow based on the work of Iliff *et al.*⁶ This could be possible if the perivascular flow compartments are different.

In this paper, we report on a benchtop experimental model of our hydrodynamic reverse transport theory for IPAD using a poly (dimethylsiloxane) (PDMS) microfluidic device. The key features of our microfluidic device are the ability to study fluid transport induced by boundary waves and to quantify fluid transport in the reverse direction of wave propagation. A schematic of our experimental setup is shown in Fig. 2. In this model, the IPAD is represented by a rectangular conduit—admittedly a great simplification

FIG. 2. A schematic showing the experimental setup to observe reverse transport. The rectangular center conduit is the model for the IPAD. Flow inside the center conduit is driven by boundary waves created by peristaltic flows inside the adjacent control channels. The peristaltic pumps create boundary motions that simulate the wave motion on the inner and outer IPAD walls due to cardiac pulsations. The center conduit in the microfluidic device is immersed in a pool of water. Fluorescent tracer particles (red dots) are injected into the center conduit and imaged at three axial positions: x_1 , x_2 , and x_3 .

of the true arterial anatomy. Yet, the same theory that supports the reverse transport observed in the rectangular conduit has already demonstrated its applicability to the annular IPAD through computational simulations.¹⁵ Here, the microfluidic experiments offer empirical evidence that validates this theory.

The flow inside the conduit is driven by wall motion that mimics arterial wall pulsations during cardiac cycles. These periodic boundary deformations are controlled by two peristaltic pumps that periodically displace fluid into cylindrical control channels on either side of the rectangular conduit. The expansion and contraction of the control channels during the peristaltic cycles deform the PDMS partitions and drive the desired boundary motion along the walls of the rectangular conduit to induce flow. The model we develop considers an incompressible Newtonian fluid and a contraction-expansion wave traveling along the conduit such that the wavelength is much larger than the conduit length, which is referred to as synchronous oscillations.²³ Physiologically, as an artery expands and contracts, the inner and outer arterial walls that bound the intramural periarterial region move in-phase. This aspect is modeled in our setup by synchronizing the two peristaltic pumps such that the partitions move in-phase with each other. Peristaltic pump A is used to simulate the deformation of the outer arterial wall surface containing smooth muscles cells and fibrous tissues, while peristaltic pump B is used to simulate the deformation of the inner arterial wall surface made of endothelial cells.

In our previous work, we discovered that reverse transport is possible purely via hydrodynamics by the superposition of forward and reverse propagating peristaltic waves.¹⁵ While we note that there are other possibilities, the presence of wave reflections is one avenue for generating reverse propagating waves. In the brain, wave reflections may be a critical mechanism for A β clearance²⁴ and are expected to originate from any change in vessel mechanical properties and arterial geometry such as branching.^{18,19} In the current microfluidic model, the forward-propagating waves are generated by the peristaltic pumps, while the reverse waves are created as reflections at an abrupt change in the control channel cross-sectional geometry.²⁵ We directly quantified the net direction and magnitude of the flow in the rectangular conduit for various wave conditions by using particle tracking velocimetry (PTV).²⁶ While here we consider only one mechanism for producing reverse propagating waves (i.e., via reflections), our results are applicable to any means for generating reverse waves since the flow is driven purely by the deformation of the boundaries.

Our experimental results validated our theory that a hydrodynamic reverse flow is possible for a specific set of boundary wave conditions. We have previously shown that the difference in the magnitudes of the reverse propagating waves on the walls enclosing the conduit must be large enough to drive a reverse flow.¹⁵ For instance, a reverse flow can be induced by having a high wave reflection on one sidewall of the conduit (e.g., control channel A side) while there is no wave reflection on the other sidewall of the conduit (e.g., control channel B side). To achieve this experimentally, we designed our microfluidic device to have an abrupt decrease in cross-sectional area in control channel A (high wave reflection) while control channel B has a constant cross-sectional area throughout (no wave reflection). The magnitude of the required wave reflection difference depends on additional wave parameters such as amplitude and distance between the conduit walls.¹⁵ To test

this theory, we fabricated different cross-section sizes of control channel A to vary the wave reflection magnitude (i.e., the magnitude of the reverse wave). We then analyzed our experimental results and validated them against computational fluid dynamics models and control volume analysis.¹⁵ This provided deeper insights into the reverse flow characteristics and IPAD.

II. MATERIALS AND METHODS

A. Microfluidic device design and fabrication

The microfluidic device consists of a rectangular conduit aligned in parallel between two cylindrical control channels. The device is made of PDMS (25:1 monomer:crosslinker; Sylgard 184; Dow Corning, Midland, MI) and is built using soft lithography and plasma bonding (Fig. 3). Molds were made by 3D printing VeroClear-RGD810 using an Object30 Pro printer (Stratasys, Eden Prairie, MN). Reusable 20G stainless steel dispensing needles (McMaster-Carr, Cleveland, OH) with a 0.93 mm outer diameter were integrated into the middle mold and defined the cylindrical control channels. The bottom mold holds the middle mold in place, while the top mold supports the uncured PDMS. After curing the PDMS, the VeroClear molds and needles were gently removed. Next, the PDMS layer was exposed to oxygen plasma (Herrick Plasma, Ithaca, NY) for 30 s and permanently bonded to a flat PDMS layer of 2.5 mm thickness with the same monomer:crosslinker ratio to enclose the rectangular channel. Capillary tubes with a nozzle diameter of 300 μ m were inserted 2.5 mm into the device on both sides of the cylindrical channels and sealed with PDMS using a monomer:crosslinker ratio of 5:1. The overall microfluidic mold design (Fig. 3) can make up to three microfluidic devices at once. The microfluidic device was connected to peristaltic pumps (Watson-Marlow 250S peristaltic pump; Watson-Marlow Pumps Group, Paramus, NJ) and a reservoir using flexible tubing (Cole-Parmer Instrument Company, Vernon Hills, IL).

FIG. 3. The microfluidic device was fabricated using a mold to make the rectangular conduit. The geometry of the rectangular mold had a height of 1.2 mm, a thickness of 0.2 mm, and a length of 25 mm. Reusable 20G stainless steel dispensing needles (McMaster-Carr, Cleveland, OH) with a 0.93 mm outer diameter were integrated into the molds.

As shown in Fig. 2, 40 ml of a water/glycerol mixture with a dynamic viscosity of 12 cSt circulates through the cylindrical control channels in two closed loops connected to two peristaltic pumps and reservoirs. The reservoirs serve to purge air bubbles from the flow system and dampen the pulse after leaving the microfluidic device to eliminate the waveforms recirculating back into the fluid circuit.²⁷ The peristaltic pumps drive forward-propagating waves along the cylindrical channels to deform the PDMS medium, inducing a boundary-driven pulsating flow in the rectangular conduit. The pumps were synchronized by positioning the rollers in identical positions and subsequently using their separate on/off switches to operate the pumps either in-phase or out-phase. The impedance of the tubing between the pumps and control channels is identical by using the same tube size and length. By submerging the rectangular conduit in a quiescent 15 ml pool of water without any pressure gradient, any flow inside the conduit is solely induced by its wall motion. The pool container was constructed by attaching the edges of 5 glass slides (25 × 75 mm 1.0 mm thick, VWR) together using industrial strength adhesive (E6000, Eclectic Products, USA). Fluorescent particles with a diameter of 3.2 μm were introduced into the rectangular conduit as PTV tracers to quantify the fluid motion under different boundary wave conditions. The wave reflection magnitudes were varied by changing the extent of the cross-section constriction near the middle of control channel A, causing the forward-propagating wave on conduit wall A to be partially reflected by the discontinuity.¹⁹ The constriction discontinuity was fabricated by inserting two dispensing needles of different diameters into each other and placing them in parallel with the rectangular conduit during PDMS molding (Fig. 4).

To produce no wave reflections, a straight and uniform cylindrical channel is embedded in the PDMS. To produce low wave reflections, a 20G needle (0.93 outer diameter) and a 29G needle (0.33 outer diameter) were joined together to form a straight cylindrical channel with a discontinuity embedded in the PDMS. To produce a medium wave reflection requires a two-step process:

FIG. 4. Diagram showing change in cross-sectional area to generate wave reflections. Diagram shows geometry for (a) no wave reflection, (b) low wave reflection, (c) medium wave reflection, and (d) high wave reflection. Before the discontinuity, the arrow moving from left to right represents the forward-propagating wave, while the arrow moving from right to left represents the reflected wave. The size of the arrow is indicative of the wave magnitude. The diagram is not drawn to scale.

(1) joining a 20G needle and a 26G needle (0.46 outer diameter) to form a straight cylindrical channel with a discontinuity embedded in the PDMS and (2) adding two thin layers of spin-coated PDMS to the smaller cross-sectional area. Each layer of PDMS was produced by injecting uncured PDMS (with a monomer:crosslinker ratio of 25:1) into the control channel, removing the excess uncured PDMS by using a spin coater set at 2500 rpm for 5 min, and then curing the PDMS in an oven. Adding thin layers of PDMS further decrease the cross-section and change the shape of the discontinuity. Finally, to produce high wave reflections, a 20 G needle and a 29 G needle were joined together to form a straight cylindrical channel with the discontinuity embedded in the PDMS and adding a single thin layer of spin-coated PDMS.

B. Flow experiments and PTV analysis

The flow in the rectangular conduit was characterized by PTV on top of an inverted epi-fluorescent Leica EL6000 microscope on which the illumination comes from a low-power mercury light bulb via a liquid light guide. A combination filter cube of band-pass filters and dichroic mirror are used to condition the illumination light into a narrow band around 480-nm wavelength. Fluorescent polystyrene tracer particles (3.2- μm diameter; 1.05 g/cm³; Thermo Scientific, Fremont, CA) were suspended in water with a volume fraction of 0.00012% and introduced into the conduit. Given water's extremely low absorption coefficient for visible light, heating of water due to the light illumination is negligible. Sinusoidal waves with a frequency of 0.5 Hz were observed on the walls and images of the particles' movements were captured using an electron multiplying CCD camera (Andor, Oxford Instruments Company, Belfast, NIR) at 24 Hz for 40 s each at locations x_1 , x_2 , and x_3 shown in Fig. 2. This imaged the particles before and after the discontinuity (x_2 and x_3 , respectively) and near the inlet (x_1). We repeated imaging at least three times at each position in the conduit.

For the PTV analysis, we identified all of the particles' center positions in each frame through peak intensity identification and Gaussian fitting of the intensities of the pixels surrounding the peaks. Next, we obtained the particles' trajectories by analyzing the list of all the particle coordinates in all frames. Trajectories were formed by unambiguous matching of nearest-neighbor particle coordinates in sequential frames. Particles were tracked over several hundred frames to elicit flow characteristics.

Drift x -velocities of the particles were obtained from their oscillatory trajectories through linear regression analysis. The particles move back and forth with each pulse. The drift velocity of a particle is defined as the slope of a least-squared fit line between the particle's x -position and time for over 20 wave periods (Sec. A in the supplementary material). A positive mean drift velocity for all particles thus indicates that the particle-carrying flow in the rectangular conduit is moving in the overall forward direction (i.e., left to right in Fig. 1, or in the direction of the peristaltic waves), while a negative mean drift velocity indicates a flow in the reverse direction (i.e., right to left, in Fig. 1, or in the opposite direction of the peristaltic waves).

C. Wall motion and wave reflection coefficient analysis

Gray-scale images of the wall positions over time were collected to reconstruct the waveform and determine the boundary

wave amplitude (a), speed (c), and wave reflection coefficient (R). The images of the wall positions were captured using a CCD camera (Q-Imaging, Surrey, BC) at 12 Hz for 28 s for the control channels, 29 Hz for 34 s for the conduit wall motion measurements, and 40 Hz for 30 s for the wave speed estimation. The Sobel edge detection algorithm in MATLAB image analysis toolbox was used to identify and track the motion of the channel edges in the microfluidic device. The periodic motions were analyzed using the Fast-Fourier transform (FFT) method in MATLAB (Sec. B in the [supplementary material](#)). We were able to identify the primary frequency of the signal, due to the peristaltic flow, and its associated wave amplitude. We note possible sources of errors originating from the CCD camera, which had a resolution of $1.818 \mu\text{m}/\text{pixel}$.

We estimated the reflection coefficient of control channel A (R_A) based on the maximum expansion of the cross-sectional diameters of control channel A (Δd_A) and control channel B (Δd_B) in a wave cycle

$$R_A \equiv (\Delta d_A - \Delta d_B) / \Delta d_B. \quad (1)$$

The maximum expansion of the cross-sectional diameter was determined relative to the corresponding channel's at-rest diameter under no peristaltic pressure. With R defined as the ratio of the reflected wave to the forward-propagating wave,²⁸ the diameter dilation attributed to the forward-propagating wave was only measured in control channel B, while the diameter dilation due to the superimposed forward-propagating and reflected waves was measured in control channel A. Thus, $\Delta d_A - \Delta d_B$ represents the change in channel cross section attributed to the reflected wave only [Sec. B, Fig. B1(a) and B1(b) in the [supplementary material](#)]. The measurements of Δd_A and Δd_B were sampled at 3 different cross sections and averaged over 14 wave cycles. The propagation of error analysis is shown Sec. C in the [supplementary material](#). It was found that all R_A values were greater than or equal to 0 due to the partially closed discontinuity³⁰ (i.e., the pressure waves in control channel A were traveling from a larger diameter cross section to a smaller diameter cross section²⁹).

We estimated the forward-propagating wave speed ($c = \Delta x / \Delta t$) by measuring the transit time delay (Δt) between the boundary wave motion along Wall B at two sites (i.e., Site 1 near the inlet and Site 2 near the outlet) separated by $\Delta x = 2 \text{ cm}$. The wave motions at the two sites were captured under the microscope. We imaged Site 1 for a 10-s duration, moved the microscope field of view to Site 2 while the CCD camera was continuously recording, and imaged Site 2 for an additional 10-s duration. This single recording of two sites allowed us to calculate Δt from the wall motions through an FFT analysis (Sec. D in the [supplementary material](#)), where it is likely that no further reflected waves were introduced into the system.³¹

D. Computational fluid dynamics (CFD) modeling

Computational modeling of the boundary wave-driven flow was conducted using the finite-volume method in ANSYS FLUENT 17.1 to supplement the experimental study. We numerically solved the Navier–Stokes and continuity equations to obtain instantaneous velocity fields inside the rectangular conduit during

periodic volume deformations. Pressures at the conduit inlet and outlet were set to zero and the fluid was initially at rest. A no-slip boundary condition was imposed on the walls while they deform. The prescribed wave functions on wall A and wall B were

$$y_A(x, t) = a \text{Re}[\exp(-i\omega t)[\exp(ikx) + R_A \exp(-ikx + 2ikL)]] + b, \quad (2)$$

$$y_B(x, t) = a \text{Re}[\exp(-i\omega t)[\exp(ikx) + R_B \exp(-ikx + 2ikL)]], \quad (3)$$

which model deformations on the inner and outer lateral surfaces of the arterial wall, respectively, separated by the width of the conduit (b). Similar to our previous work,¹⁵ we assumed that the waves take the form of sinusoidal functions with the same wave number (k), amplitude (a), and angular frequency (ω). We set $\omega = 0.51 \text{ Hz}$, $a = 5.4 \mu\text{m}$, $\lambda = 0.94 \text{ m}$, $L = 0.0125 \text{ m}$, and $b = 200 \mu\text{m}$ in our simulation. The forward-propagating peristaltic wave is partially reflected at $x = L$. The reflected wave propagates in the negative x -direction with a smaller amplitude (since $R_{A,B} < 1$), and we assumed no further wave reflection occurs at $x = 0$. The reflection coefficient, $R_{A,B}$, is an independent non-dimensional parameter and depends on the local wave medium discontinuity due to changes in local geometric and mechanical properties.³²

To accommodate the deforming rectangular conduit, we used the diffusion-based smoothing method to update the finite volume mesh during each time step.¹⁵ In ANSYS FLUENT, this method moves the mesh nodes in response to the displacement of the boundaries by solving the diffusion equation for mesh velocities. The simulations were performed for a time duration of eight wave periods. The average velocity, u_{avg} , over a wave period, τ , was obtained by

$$u_{\text{avg}} \equiv \frac{1}{S_{\text{initial}}} \int_t^{t+\tau} \left\{ \int [\vec{u}(x=0, y, t') \cdot \hat{x}] dS \right\} dt', \quad (4)$$

where S_{initial} is the initial cross-sectional area of the conduit. All simulations show u_{avg} during the seventh and the eighth wave periods differed by less than 0.01%, which suggests that during the eight periods, the flow had reached a periodic state.

Furthermore, we conducted a mesh size and time step sensitivity analysis to ensure that our solution is independent of these computational parameters. Our mesh consisted of 70 856 nodes and we chose a time step size of $\Delta t = 0.02 \text{ s}$. The mesh sensitivity of the simulations was determined by varying the number of nodes in the domain and comparing the average velocity. For the same rectangular volume, the number of nodes was varied from 26 271 to 159 426 while maintaining $\Delta t = 0.02 \text{ s}$. Within this range of grid resolution, average drift velocity was found to change by less than 0.0068%, which indicates that the lower mesh density was sufficient. The time step size sensitivity was evaluated by varying Δt between 0.025 and 0.01 s and comparing the average velocity while keeping the number of nodes fixed to 70 856. We confirmed that the average drift velocity changes by less than 0.096%, which suggests that a relatively larger time step size can be used for computational efficiency.

We also calculated the pathlines to obtain additional insights into the flow characteristics. Pathlines were obtained by tracing the

FIG. 5. Forward flow was experimentally observed when there are no wave reflections. x -displacement vs. time of tracer particles for [(a)–(c)] $R_A = 0$, $R_B = 0$ and [(d)–(f)] $R_A = 0.99 \pm 0.04$, $R_B = 0$. The particles were imaged at x_1 [(a), (d)], x_2 [(b), (e)], and x_3 [(c), (f)]. Each line represents the time-averaged drift velocity of a tracer particle.

trajectory of fictitious particles' in the flow using the velocity field data from the FLUENT simulations. A linear interpolation method of particle velocities was adopted for the numerical integration in the MATLAB environment. The accuracy of this tracing method was deemed sufficient since the drift velocity of 578 tracers obtained throughout the domain consisting of 70 856 nodes and 159 426 nodes was found to, on average, vary less than 1.2%.

E. Control volume analysis

The control volume analysis was reported in our previous study.¹⁵ It is provided here for comparison with the CFD model and experiments. The control volume analysis was used to calculate the overall average flow velocity and provides insight into the generation of a net reverse flow. The average velocity from the control volume analysis solution is

$$u_{avg} \approx \frac{Q_{cv}}{S_{initial}} \approx \frac{\frac{1}{S_{initial}} \int_0^{\tau} \frac{dV}{dt} \frac{1}{1 + \alpha^{1/2}} dt}{1 - \frac{1}{2\beta} \int_0^{\tau} \frac{S_0(t)}{S_0} dt \int_0^{\tau} S_0 \alpha^{1/2} dt}, \quad (5)$$

where Q_{cv} is the net volume transported per wave period, dV/dt is the instantaneous volume rate of change of the conduit, and α is the cross-sectional area ratio, which vary in time, between $x = 0$ and $x = L$. S_0 represents the cross section area at $x = 0$, while $S_{initial}$ is the value of S_0 at $t = 0$. The momentum-flux correction, β , is set to 1.50 for a rectangular geometry. Physically, Eq. (5) shows that a reverse flow can be generated due to the integrated effect of the rate of change of the conduit volume and the cross sectional areas of the inlet and outlet. The rate of change of the rectangular volume provides the driving force for fluid motion, while the relative sizes of the inlet/outlet openings determine the instantaneous flow direction.

Therefore, an overall reverse flow can be obtained when $\alpha > 1$ for an expanding volume and when $\alpha < 1$ for a contracting volume.¹⁵

III. RESULTS

A net flow in either the forward or the reverse direction depends on the boundary wave conditions. For no boundary waves, we confirmed that the particles remained almost stationary. Our previous theoretical and numerical work showed that the conditions necessary to induce a reverse flow are (i) $R_A \neq R_B$ and (ii) $|R_A - R_B|$ must be sufficiently large.¹⁵ In contrast, wave parameters that do not satisfy these conditions result in a net forward flow. Here, our experimental results confirm that satisfying these conditions will generate a reverse flow.

A. The effect of $R_A \neq R_B$ on the flow direction

Figure 5 shows the tracer particles' drift in the x -direction versus time for the case of no wave reflections on the conduit walls, $R_A = 0$, and $R_B = 0$ [Figs. 5(a)–5(c)], and for the case of a high wave reflection on wall A and no wave reflections on wall B, $R_A = 0.99 \pm 0.04$ and $R_B = 0$ [Figs. 5(d)–5(f)]. Through a linear regression

TABLE I. Overall drift velocities (u_{avg}) in the x -direction under various values of R_A with $R_B = 0$.

R_A	Axial position		
	x_1 ($\mu\text{m/s}$)	x_2 ($\mu\text{m/s}$)	x_3 ($\mu\text{m/s}$)
0	0.28 ± 0.15	0.30 ± 0.08	0.46 ± 0.22
0.43 ± 0.04	0.15 ± 0.20	0.06 ± 0.08	0.10 ± 0.07
0.72 ± 0.04	-0.20 ± 0.16	-0.74 ± 0.20	-0.19 ± 0.11
0.99 ± 0.04	-0.85 ± 0.43	-0.84 ± 0.37	-0.75 ± 0.23

FIG. 6. Forward flow was experimentally observed when incident waves are moving out-of-phase despite wave reflections. Drift x -displacement vs. time at (a) x_1 , (b) x_2 , and (c) x_3 when the incident waves are moving out-of-phase between the conduit walls and $R_{A,B} = 0.99 \pm 0.04$, 0. Each line represents the drift of one particle. The results show that the phase angle between the conduit wall motions contributes to the net particle direction. When the walls are in-phase of each other [Figs. 5(d)–5(f)], particles are transported in the reverse direction. On the other hand, the particles move in the forward direction when the walls periodically deform 180° out-of-phase from each other, as shown here. This is despite the presence of high wave reflection. The overall drift velocities are $2.27 \pm 0.66 \mu\text{m/s}$, $1.37 \pm 0.686 \mu\text{m/s}$, and $0.872 \pm 0.335 \mu\text{m/s}$, at x_1 , x_2 , and x_3 , respectively.

analysis, the slope of each line in Fig. 5 represents the time-averaged drift velocity of a tracer particle in the axial flow. An overall drift velocity was calculated by averaging all of the particles identified in 3 or more image sets acquired at each axial location (i.e., x_1 , x_2 , and x_3 in Fig. 2). A positive value for the x -displacement (or velocity) indicates that the particles are moving in the same direction as the peristaltic flow in the control channels, while a negative x -displacement indicates that the particles are moving in the reverse direction. The drift velocities vary among the tracer particles because of the no-slip wall boundary condition and the non-uniform cross-sectional velocity profile inside the conduit. Nevertheless, the case of $R_A = 0$, and $R_B = 0$ [Figs. 5(a)–5(c)] shows a net forward flow, while the case of $R_A = 0.99$ and $R_B = 0$ [Figs. 5(d)–5(f)] shows a net reverse flow. Table I summarizes the measured drift velocities from our experiments for various values of R_A with $R_B = 0$.

In addition, we performed an experiment with high wave reflections on both walls of the conduit (i.e., $R_A = R_B \approx 0.9$) and measured a net forward flow. In all, the results support our theory

that if the boundary deformations on the conduit walls are similar (i.e., $R_A \approx R_B$ and $R_{A,B} > 0$), the flow would be driven in the forward direction despite the presence of wave reflections.¹⁵

We also note that phase differences between the boundary waves can play a secondary role in determining the direction of the flow.¹⁵ We performed an additional experiment with the forward-propagating waves on the walls being 180° out-of-phase of each other and measured a net forward flow, despite the fact that $R_A = 0.99 \pm 0.04$ and $R_B = 0$ (Fig. 6).

B. The effect of increasing $|R_A - R_B|$ on the flow magnitude

To highlight the importance of the key parameters on the overall drift velocity, the following results mainly focus on the conditions where a reverse flow is possible, i.e., $R_B = 0$ and $R_A > 0$. We varied R_A by changing the geometry of the control channel A discontinuity and measured the overall drift velocities (Table I).

FIG. 7. Flow transitioned to the reverse direction as wave reflection magnitude increased. Reflection coefficient (R_A) vs. average drift velocity (u_{avg}) for $R_B = 0$ at three different axial positions (a) x_1 , (b) x_2 , and (c) x_3 . Blue circle markers represent experimental measurements, red triangle markers represent computational simulation results, and black solid lines represent control volume analysis (CVA) calculations. Positive u_{avg} indicates tracer particles moving in the forward direction, while negative u_{avg} indicates particles moving in the reverse direction. The error bars represent the standard deviation (spread) of the measured particle drift velocities as shown in Sec. A in the supplementary material.

FIG. 8. Experiment shows percentage of tracer particles moving in the reverse direction increased as wave reflection magnitude increased. Reflection coefficient (R_A) vs. percentage of tracer particles moving in the reverse direction. All data were obtained under $R_B = 0$.

The tested reflection coefficients include $R_A = 0.43 \pm 0.04$ (low wave reflection), 0.72 ± 0.04 (medium wave reflection), and 0.99 ± 0.04 (high wave reflection). It was found that at low values of R_A , the flow in the conduit is in the forward direction. In this regime,

increasing R_A (or equivalently increasing $|R_A - R_B|$) decreases the overall drift velocity until the flow starts changing into the reverse direction, as shown in Fig. 7. Our experimental measurements suggest that the flow changes direction at $|R_A - R_B| \approx 0.5$, which supports the conclusion of our previous theoretical work.¹⁵ Further increasing the values of $|R_A - R_B|$ beyond 0.5 enhances the magnitude of the reverse flow in the conduit.

The drift velocities obtained from our CFD simulations and control volume analysis are consistent with our experimental results, as shown in Fig. 7. For these calculations, the measured wave parameters (frequency 0.51 Hz, amplitude $5.4 \mu\text{m}$, and wavelength 0.94 m) and conduit geometry ($L = 0.0125 \text{ m}$ and $b = 200 \mu\text{m}$) were substituted into Eqs. (2) and (3) to prescribe the boundary motions. For the simulation, the net flow is indeed positive when $R_A = 0$; however, the predicted magnitudes are quite small. Figure 7 shows zoomed-in views at $R_A = 0$. This validation allows us to use modeling results to complement the experimental measurements.

We next considered the percentage of tracer particles moving in the reverse direction as R_A increases while $R_B = 0$. For no wave reflections, nearly all of the particles are transported in the forward direction. As R_A increased, the percentage of particles experimentally measured to be moving in the reverse direction increased, as shown in Fig. 8 (blue circular markers). For high wave reflections, nearly all of the particles moved in the reverse direction. A similar trend is also observed in our CFD simulations

FIG. 9. Simulation shows percentage of tracer particles moving in the reverse direction increased as wave reflection magnitude increased. Initial position of particles and their overall displacement for (a) $R_{A,B} = 0, 0$, (b) $R_{A,B} = 0.10, 0$, and (c) $R_{A,B} = 0.99, 0$. The blue markers indicate that the particles are moving in the positive x -direction, while the red markers are moving in the negative x -direction. The overall direction of the particles was determined by the sign of their drift velocity for 200 wave cycles. In our numerical simulations, all the particles move in the forward direction in the absence of wave reflections, as shown in (a). The presence of weak wave reflections ($R_A = 0.10$) along one side of the conduit wall is enough to drive the majority of the particles in the reverse direction, while the remaining particles are moving in the forward direction at two distinct regions, as shown in (b). These distinct regions reduce in size as R_A increases, as shown in (c).

FIG. 10. Pathlines in the simulation show tracer particles in the conduit undergoing an overall reverse flow. The reflection coefficients are $R_A = 0.72$ and $R_B = 0$. There exists a reverse transport path (from upper-right to lower-left of the figure) that separates two regions with converse trajectories (dotted lines). Note that for clarity, the vertical axis has been stretched.

(red triangle markers, Fig. 8) based on the trajectories of 578 simulated tracer particles. These particles were placed throughout the computational domain at the beginning of the simulations and their displacements were recorded for 200 wave cycles (Fig. 9).

For no wave reflections, almost all particles drift in the forward direction. Moreover, our simulations show that particles near the center of the conduit move faster than the particles near the wall because of the no-slip boundary condition. For high wave reflections, our simulations show that an overall reverse flow consists of a reverse-moving path separating two regions with converse trajectories (Fig. 10, dotted arrows) inside the conduit. These results show a streaming flow (i.e., net flow) similar to the phenomenon reported by Hydon and Pedlet (1993). While they showed that channels whose walls remain parallel but oscillate transversely can generate a streaming flow for Peclet and Reynolds numbers greater than unity,³³ our experiment suggests that streaming flow can be achieved due to wave reflections when the Peclet number and Reynolds number is much less than unity (Sec. E in the [supplementary material](#)). The particles in the reverse-moving path bypass the two regions and are transported across the entire channel towards $x = 0$.

We did observe some considerable differences between simulation and measurements of the percent of particles flowing in the reverse direction as a function of the amplitude of the reflected wave (Fig. 8). There are several factors that may have contributed to this: (1) we only used a sine wave at a single frequency in our numerical models, (2) we used a simple model of the wall deformations, and (3) the experimental flowrate was not directly calculated (only average velocity of particles); numerically, the average velocity was based on the flow rate. Nevertheless, the observed and simulated trends are in the same direction.

IV. DISCUSSION AND CONCLUSIONS

Our experimental model is a simplification of IPAD in the brain and attempts to capture the low Reynolds and Womersley number fluid dynamics occurring in the arterial wall. Section E in the [supplementary material](#) shows the similitude of the experiment,

simulation, and the predicted values one would expect physiologically. Indeed, the geometry of the IPAD resembles more of an annulus rather than a rectangular conduit. Although the flow magnitude is dependent on the geometry of the model, it is expected that the characteristics of the reverse propagating waves determine the flow direction regardless of whether it is an annular or rectangular geometry. The simulation is a simplification of the experiment: the flow was modeled as 2D with a sinusoidal boundary wave frequency of 0.51 Hz only. Nevertheless, we observed that a viscous reverse flow driven by boundary waves through a thin conduit is possible, confirming our theory of potential ISF transport through a single arterial basement membrane.

We have discovered that a boundary wave-driven reverse flow can be induced under the conditions that $R_A \neq R_B$ and $|R_A - R_B|$ are sufficiently large. We contend that one possible source of the forward and reverse propagating boundary waves on the artery wall is from the heart pulsation. Wave reflections likely occur at arterial junctions, or the sites of arterial branching, but may also occur wherever the mechanical properties of the bounding membranes abruptly change. Reflection coefficients highly depend on the conduit geometry, such as the cross-sectional area ratio (the ratio of the sum of the area of daughter vessels to that of the mother).²⁹ Given that the IPAD conduit wraps around the lumen and takes an annular shape, its inner and outer boundaries must have distinct branching geometries that could result in $R_A \neq R_B$. We also speculate that sufficiently large $|R_A - R_B|$ could be satisfied by the differences in the mechanical properties of the membranes bounding the IPAD region. The magnitudes of wave reflection coefficients also highly depend on the solid properties, such as rigidity and elasticity, of the transmitting medium. The annular IPAD region is bounded from the inside by the endothelial cell layer of the lumen (and likely its basement membrane) and from the outside by smooth muscle cells and fibrous tissues [and its basement membrane(s)]. Given the significant differences in the cell and tissue types that form these boundary surfaces, large values of $|R_A - R_B|$ would conceivably be possible. We also found that the magnitude of reverse transport is correlated with $|R_A - R_B|$. Thus, it is also theoretically feasible that age-related changes in the mechanical properties of the endothelial cells, basement membranes, smooth muscle cells, and fibrous tissues result in decreases of $|R_A - R_B|$ that reduces reverse transport of A β .

The pathlines constructed within our computational model for a reverse flow with $R_A = 0.72$ and $R_B = 0$ reveal that perhaps not all solutes are transported in the reverse direction out of the conduit. Some of the solutes could slowly move back-and-forth near the conduit walls. It is also conceivable that A β trapped in these regions gradually adhere to the arterial basement membrane surfaces over time, leading to the onset of cerebral amyloid angiopathy.

Our model also indicates that our geometric arrangement does not lead to a 100% reverse transport. We can achieve approximately 85% reverse transport of particles at best (Fig. 8). However, 100% of solute transport in the reverse direction can be attained by occluding a fluid packet and squeezing it in the reverse direction, as illustrated in Fig. 11. We can occlude part of the channel if the wave amplitude is large and the conduit is small enough to cause an occlusion. Any combination of R_A and R_B , except $R_A = R_B$, can create a reverse flow with 100% of the particles when the inner and

FIG. 11. Schematic showing the sequence of occlusion-enhanced reverse transport. The standing wave is formed by superimposing a forward and reverse propagating wave. As the waves come into contact with each other, they form an enclosed fluid packet and transport it in the reverse direction, where τ is defined as the wave period. The set of parameters for this example is $R_A = 0$, $R_B = 1$, $a = 10 \mu\text{m}$, $\omega = 2\pi$, $\lambda = 0.00625 \text{ m}$, $L = 0.0125 \text{ m}$, and $b = 10 \mu\text{m}$.

outer lateral surfaces are close enough to trap and transport a fluid packet in the reverse direction. The intent of Fig. 11 is to show an example of extremely efficient reverse transport with enclosed packets. This condition would not match to the exact condition in the brain. This occlusion-enhanced transport may be physiologically plausible in the brain, since a single layer, between layers of smooth muscle cells, of the IPAD region is 150 nm thick¹⁰ and the pulse wave amplitudes in the brain can be 30%–40% of the arteriole lumen diameter.³⁴

The vascular walls consist of many constituents such as endothelium, elastic and fibrous tissues, and smooth muscle cells.³¹ This suggests that fluid within the arterial walls is transported in a porous (and possibly active) medium, which consist of the combination of active smooth muscle cells and extracellular matrix. It has been suggested that this structure would generate a flow resistance in the perivascular space that may be too large for IPAD.³⁵ The IPAD is simplified as an open channel in our experimental model to highlight the importance of the key parameters. One may presume that the small pores would increase fluid flow resistance and therefore decrease the overall transport. However, a porous structure may instead provide a favorable medium for the occlusion-enhanced transport. As the boundary waves deform the arterial walls, periodic opening and closing of the pores could easily create local occlusions and fluid trapping that lead to reverse

transport. Clearly, this mechanism relies on the elasticity of the arterial wall structures and could suffer significant reduction if the vascular walls become stiffer due to aging.

The exact mechanism of A β clearance from a healthy brain remains elusive. Since the lack of A β clearance out of the brain is associated with AD, understanding the forces driving this transport may advance diagnostic metrics and therapeutic schemes for AD. In this paper, we validated our reverse transport theory through bench-top experimentation. We contend that this mechanism is a potential candidate for explaining IPAD of A β clearance. Moreover, we conducted computational simulations that support our findings and identified the key boundary wave conditions needed for a net reverse transport driven by peristaltic waves propagating only in the forward direction. This theory offers a potential explanation of the biomechanical causes for A β clearance along the cerebral IPAD region.

SUPPLEMENTARY MATERIAL

See the [supplementary material](#) for details on: the calculation of the drift velocity for a tracer particle (Sec. A), the measurement of boundary wave motion inside the microfluidic device (Sec. B), the propagation of error analysis (Sec. C), measurements of wave speed (Sec. D), and the comparison of the experimental, numerical, and physiological parameters (Sec. E).

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