Enhanced H-filter based on Fåhræus-Lindqvist effect for efficient and robust dialysis without membrane

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A novel microfluidic device for highly efficient and robust dialysis without membrane is highly desired for the development of portable or wearable microdialyzer. Here we report an enhanced H-filter with pillar array based on Fåhræus-Lindqvist effect (F-L effect) for highly efficient and robust membraneless dialysis of simplified blood for the first time. The H-filter employs two fluids laminarly flowing in the microchannel for continuously membraneless dialysis. With pillar array in the microchannel, the two laminar flows, with one containing blood cells and small molecules and another containing dialyzate solution, can form a cell-free layer at the interface as selective zones for separation. This provides enhanced mixing yet extremely low shear for extraction of small molecules from the blood-cell-containing flow into the dialyzate flow, resulting in robust separation with reduced cell loss and improved efficiency. We demonstrate this by first using *Chlorella pyrenoidosa* as model cells to quantitatively study the separation performances, and then using simplified human blood for dialysis. The advanced H-filter, with highly efficient and robust performance for membraneless dialysis, shows great potential as promising candidate for rapid blood analysis/separation, and as fundamental structure for portable dialyzer. © 2015 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4927574>]

I. INTRODUCTION

Chronic kidney disease is a serious disease associated with premature mortality, decreased life quality, and increased health-care expenditures.¹ It is one of the three causes including HIV/AIDS and diabetes account for more than a half a million deaths, and its global age-standardised death rates have increased significantly from 409 000 in 1990 to 956 000 in 2013.² Untreated chronic kidney disease can result in end-stage renal disease (ESRD). In 2006, more than 1.6×10^6 ESRD patients have to receive dialysis treatment, and this number grows at a rate of 7% per year.³ Hemodialysis is a standard therapy⁴ for ESRD, which can extracorporeally remove waste products such as creatinine, urea, and free water from blood. During the hemodialysis, the waste products diffuse from the blood into counter-current dialyzate across a semipermeable membrane via concentration gradient, etc.^{3,5,6} However, the annual mortality for the hemodialysis patients is still up to 20% (Ref. 6) due to increased cardiovascular risk^{5,7} and high depression.^{7,8} Moreover, most ESRD patients need 3 times hospital/clinic visits per week

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for a lifetime because of the low-efficient hemodialysis;^{3,9–12} this causes great inconvenience as well as a heavy economic burden. Therefore, development of more efficient dialyzer to improve patient outcomes, especially for the personalization and daily treatment, is of great importance.⁴ Microfluidic technique, which enables controllable manipulation of flows within the low Reynolds number in the microchannel of a miniaturized device,^{13,14} provides efficient mass transfer and extremely low shear for manipulating biomolecules for myriad biomedical applications such as analysis and separations.^{15,16} Thus, microfluidic techniques have been identified as the key to develop state-of-the-art dynamic devices for biomedical applications,^{6,13,17,18} which is promising for development of portable and delicate hemodialyzer. Therefore, combination of microfluidic techniques with hemodialysis for developing portable or wearable dialyzer will be a big breakthrough in ESRD therapy.

Typically, two strategies are used for microfluidic-derived hemodialysis, including the dialysis with and without membrane in the microfluidic channels. Membrane-integrated microfluidic hemodialysis is achieved by implanting microchannels with commercial ultrafiltration membranes,^{3,19} nanopore membranes,^{9,20} electrospinning nanofiber web,¹² or even living membranes incorporating renal tubule cells as the selective solid barriers for dialysis.^{10,21,22} However, these platforms suffer from complicated process for assembling the device and membrane, easy clogging or fouling for the membranes, and troublesome process for membrane recycling. By contrast, membraneless microfluidic hemodialysis, with two directly contacted fluids that laminarily flow in microchannel, allows small molecules to pass through the liquid interface easily. Thus, the membraneless dialysis is an improved process with easier fabrication and recycling process,¹⁵ better biocompatibility, higher transport rate, and no clogging, as compared with the membrane-integrated ones. However, as far as we know, there is no report on applying membraneless dialysis for robust hemodialysis in the microfluidic device up to now.

Generally, microfluidic devices for membraneless dialysis usually employ H-shaped microchannels as the filter.^{15,23–27} The H-filter,^{15,23,24} with two laminar flows in one microchannel, allows continuous extraction of small molecule analytes from flow that contains large particles (e.g., blood cells) for purification and extraction. Nevertheless, the directly contacting interface between the two laminar flows in H-filter may break upon disturbance, leading to mix of the two flows and loss of the large molecules.²⁴ For example, the H-filter used for protein separations still suffers 5%–30% loss of large proteins at different flow rates.²⁴ Moreover, solute exchange in H-filter relying on only diffusion is slow compared with cases involving additional convection.²⁸ The robustness and efficiency of separation and extraction in H-filter can be improved by structure or surface modification. An asymmetric inlet and outlet of H-filter is designed as artificial glomerulus, which keeps robust by recovering additional dialyzate.²⁵ Parallel countercurrent laminar flows are produced by the selective surface modification of microchannel wall,²⁹ which enhances the extraction efficiency of a cobalt complex by 4.6 times higher than conventional cocurrent flows.²⁶ However, this device can only perform at total flow rate of maximum $20\ \mu\text{l min}^{-1}$. An improved H-filter based on sheath flow (three-layer laminar flow) with higher efficiency has been developed.²⁷ It is also potential for robust hemodialysis if strong cell lateral migration happens, but the migration only occurs at carefully selected flow conditions yet to be found. Up to now, its red blood cell loss is still above 10%.^{30,31} Till now, the improved H-filters either suffer the cell loss or operate at low efficiency during the membraneless dialysis. Therefore, new microfluidic devices with simultaneously highly efficient and robust separation and extraction especially for membraneless hemodialysis are still highly desired.

In 1930, Fåhræus and Lindqvist discovered that when blood passed through capillary with diameter from 10 to $300\ \mu\text{m}$, a cell-free layer formed near the capillary wall, and the viscosity of blood fell down with the decrease of diameter.³² This phenomena was called Fåhræus-Lindqvist effect (F-L effect), which contributed to the later discovery of hydrodynamic inertial lift force^{33,34} and deformation-induced non-inertial lift force^{35,36} in the microchannels. Inspired by the F-L effect,^{32,35} here we design and develop an enhanced H-filter with pillar array along the centerline of the microchannels for highly efficient and robust membraneless dialysis. The existence of the pillar array in H-filter does not affect the formation of the continuous laminar

interface between two fluids, but rather it helps to form a cell-free layer. In detail, the red blood cells obtain hydrodynamic inertial lift force (mainly shear-gradient and wall-effect lift forces)³⁷ and non-inertial lift force caused by shear deformation when passing around a pillar in the laminar interface. As a result, the red blood cells flow towards the channel wall, leading to the formation of a cell-free layer near the pillar. The cell-free layer develops wider due to periodic lift force induced by the pillar array, which could decrease the cell loss and guarantee the robust dialysis. Meanwhile, the extraction of small molecules in blood waste such as urea will be enhanced by pillar-induced chaotic mixing,^{38–41} which has potential for high efficient dialysis without membrane. During the dialysis, the reliable collection of blood cells and chaotic mixing are observed in the enhanced H-filter. Therefore, the proposed enhanced H-filter is highly potential and promising for the analysis or separation of blood, and even as the fundamental structure of portable dialyzer.

II. EXPERIMENTAL

A. Microfabrication

Microchannels of the enhanced H-filter are fabricated by standard soft-lithography techniques⁴² using SU-8 2035 (Microchem) and patterned with polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning). To optimize the design of enhanced H-filter, the straight microchannels with a single pillar or pillar array which with different pillar diameters and pillar intervals are designed and fabricated. The schematic illustrations of straight microchannels with a single pillar or pillar array are shown in Fig. S1 in the supplementary material.⁵¹ The width and depth of all the tested microchannels are about 200 μm and 24 μm , respectively. The structure and dimension of the enhanced H-filter with a pillar array are observed by a scanning electron microscope (SEM; G2 Pro, Phenom).

B. Cell and solution preparation

To better observe and visualize F-L effect, *Chlorella pyrenoidosa* (CP) cells with autofluorescence are used as the blood model in order to visualize and quantify the F-L effect, and optimize the design of microchannels. CP cells are grown in the Bold's Basal Medium (BBM) with concentration of about 3×10^6 particles per millilitre, which are cheap and easy to deal with. The fluorescent image shows that the size of CP cells is approximately 3–5 μm (Fig. S2(a) in the supplementary material⁵¹), which is close to the size of red blood cell. The similar size indicates that the CP cell is a suitable candidate of blood model.

Isotonic solutions of 1.49 wt. % KSCN and 1.24 wt. % FeCl_3 are used to visualize and quantify the mixing efficiency of small molecules in the microchannel since the product $[\text{Fe}(\text{SCN})_n]^{(3-n)}$ ($n = 1-6$) takes on blood red. For the dialysis experiment, isotonic solutions of 1.24 wt. % FeCl_3 solution containing CP cells and 1.49 wt. % KSCN are used. CP cells are centrifuged (TGL-16C, Shanghai Anting Scientific Instrument Factory) at 5000 rpm for 5 min, and then dispersed into FeCl_3 aqueous solution. The solutions containing cells are filtered by a cell strainer with 1000 Mesh before use.

For the hemodialysis experiment, FeCl_2 solution containing blood cells and $\text{C}_{12}\text{H}_8\text{N}_2$ solution are used as blood and dialyzate. Here FeCl_2 solution rather than FeCl_3 solution is used as the indicator during the dialysis, because the oxidizing FeCl_3 damages blood cells, resulting in precipitation. Before dialysis experiments, the whole blood with anticoagulant (Sichuan Neo-Life Stem Cell Biotech) is washed by 0.9 wt. % NaCl solution with 4 times of volume and then centrifuged for 10 min at the rate of 4000 rpm (Sorvall Biofuge Primo R, Thermo Scientific) for 5 times to remove the white cells and fibrinogen which will adsorb on PDMS surface.⁴³ After removing the supernatant, the washed blood is diluted to the original volume with 0.9 wt. % NaCl solution. Subsequently, the blood is further diluted with FeCl_2 isotonic solution to 11 times of volume before use. Because the whole blood source is scarce, the diluted solution of red blood cells is used. The FeCl_2 isotonic solution is composed of 1.30 wt. % FeCl_2 and 0.02 wt. % ascorbic acid for antioxidation. The solutions containing blood

cells are also filtered by the cell strainer before use. The dialyzate is prepared by dissolving $\text{C}_{12}\text{H}_8\text{N}_2\cdot\text{H}_2\text{O}$ (0.15 w/v %) into 0.9 wt. % NaCl solution at 80 °C.

C. Set up and characterization

The F-L effect and chaotic mixing in the microchannels are observed by a fluorescent microscope (DM 4000, Leica) under an I3 filter set (BP 450–490) and the bright field. The fluids are pumped into the microchannels by syringe pumps (PHD 2000, Harvard Apparatus). The magnetic stirring is used to maintain cell suspension in the syringe referring to the method in Ref. 44. All the experiments are carried out at 25 °C. The images recorded by microscope are analysed and optimized by using ImageJ 1.47t (<http://imagej.nih.gov/ij/>) and MATLAB R2009a (The MathWorks, Natick). Origin Pro 8.5 (OriginLab, Northampton) is used to plot multiple colour fill surfaces.

III. RESULTS AND DISCUSSION

A. Quantification of F-L effect and optimization of microchannel

1. Effect of pillar shape

When a suspension flows around a pillar in microchannel, the pillar-induced shear-gradient and wall-effect lift forces will push cells away from pillar surface. To understand the subsequent separation, we investigate experimentally the roles of pillar shape in F-L effect. The setup is simply a straight channel with a single pillar, as schematically shown in Fig. S1(a) in the supplementary material.⁵¹ In the experiment, a thin strip of non-fluorescent wake (cell-free layer)⁴⁵ could be observed downstream of the pillar. To easily quantify the F-L effect, we measured the wake width (“ W_2 ” in Fig. 1(a-1)) at about $\Delta x = 100\ \mu\text{m}$ from the pillar in the downstream, while the size of pillar as “ W_1 ” (Fig. 1(a-1)) in width. Obviously, the wider the wake (“ W_2 ”), the more significant the F-L effect, which is helpful to develop robust membrane-less hemodialysis. Totally, we choose seven pillar shapes to observe, which are circle, ellipse, triangle of round top, rhombus, triangle, square, a long rectangle with round ends (#1–#7 in Fig. 1(a)). In other words, they are seven combinations of various front and rear curvatures of pillar surfaces. In this logic, the first six shapes are short, so there is a short period available for curvature transition from the front to the rear, which provides different shear-gradient and wall-effect lift forces. By comparison, the transition of the seventh shape is pretty much long. All pillars are held as the same width $80\ \mu\text{m}$, such that the smallest gaps between pillars and channel walls are identical. Two flow rates are chosen in our experiment: $200\ \mu\text{l h}^{-1}$ and $5000\ \mu\text{l h}^{-1}$ to provide a high contrast in inertia of fluid. The fluorescent images of wakes induced by pillars with different shapes in the case of $5000\ \mu\text{l h}^{-1}$ are shown in Figs. 1(a-2)–1(a-8). All the wakes visualized as the dark or non-fluorescence strip are intuitively observed in the right panels with enlarged fluorescent images. Fig. 1(b) summarizes the relationship between width of wake and pillar shape at $200\ \mu\text{l h}^{-1}$ and $5000\ \mu\text{l h}^{-1}$. The difference among wake width of the short pillars #1–#6 can be ignored at both cases, which is caused by the limited contacting time of CP cells and pillar surface for fast curvature transition. By contrast, the wake width of the long pillar (#7) is significantly large relative to that of the other pillars (#1–#6). It is attributable to the pillar #7 has the much larger axial length ($1080\ \mu\text{m}$) than the pillars #1–#6. Such a long axial length provides a long term journey for cells to experience the lift force near pillar surface. Besides, because the shear gradient near the pillar wall is greater in channel with a long pillar, the particles will rotate and obtain rotation-induced weaker inertial lift force³⁴ just like the Magnus effect.³⁵ This result can be further confirmed by the cells adhesion to pillar walls. For short pillars (#1–#6), there are bright spots often at the pillar surfaces, as shown in Figs. 1(a-2)–1(a-7), indicating that the cells still run closely to the surface even at the rear end.⁴⁶ However, no CP cells are found on the pillar #7 (Fig. 1(a-8)), manifesting that the cells are far enough from wall of pillar #7. In other words, the slow transition from the front surface to the back helps develop a thick cell-free layer, and cells above this layer flow in parallel to the streamline driven by their inertia.⁴⁶ Generally, the contribution of lift

FIG. 1. Effect of pillar shape on F-L effect. (a) Schematic illustration of straight microchannel with a single pillar. Solution A is *CP* cell solution (a-1), and the fluorescent images of wakes (W_2) induced by pillars with different shapes at the flow rate of $5000 \mu\text{L h}^{-1}$ (a-2)–(a-8). The *CP* cell solution is used for visualization of F-L effect. The width of different shapes (W_1) is about $80 \mu\text{m}$, and measured at the downstream distance about $\Delta x = 100 \mu\text{m}$ away from the pillar. Scale bars in left panels of Figs. 1(a-2)–1(a-8) are $100 \mu\text{m}$, and that in the right panels with enlarged images are $20 \mu\text{m}$. (b) Effect of the pillar shape on the width of wake at the flow rate of $200 \mu\text{L h}^{-1}$ and $5000 \mu\text{L h}^{-1}$.

force to “ W_2 ” due to the curvature of pillar surface is nearly the same according to our observation on all short pillars. The microchannel with the shape of pillar #7 can run longer time without clogging, which provides valuable guidance for the following design of channels.

2. Effect of the pillar density

The pillars intervals are where the filter works, and small molecules are supposed to transport into the dialysate as many as possible. The longer the intervals are, the more molecules will leave from the cell suspension. What will happen to the cell-free layer in terms of “ W_2 ” is studied in the straight channel with a pillar array (Fig. S1(b) in the supplementary material⁵¹). The pillar of circle (#1) with diameter of $80 \mu\text{m}$ is used to form the pillar array. Although pillar of shape #7 produces a large width of wake, the long shape is not proper for the fabrication of pillar array with large cardinality. The pillar of circle (#1) is representative of the short pillars, and more than that, it can provide a high mixing efficiency of small molecules.³⁹ In our

experiment, the pillar interval (ΔL) varies from $200\ \mu\text{m}$ to $1000\ \mu\text{m}$. Here, “ W_2 ” refers to the width of wake at about $\Delta x = 100\ \mu\text{m}$ away from the last pillar in the downstream. The pillar array’s working distance in channel is kept $7000\ \mu\text{m}$ with various intervals of 1000 , 500 , 400 , 300 , and $200\ \mu\text{m}$. The pillar array is coded as No.- W_1 - n - ΔL as shown in Fig. 2(a), where “No.” refers to the type of pillar shape shown in Fig. 1, “ W_1 ,” “ n ,” and “ ΔL ” stand for the width, number, and interval of pillars in the array, respectively. The results of “ W_2 ” at flow rates of 1000 , 5000 , and $9000\ \mu\text{L h}^{-1}$ (Fig. 2(b)) show that at the same flow rate, “ W_2 ,” increases with the density of pillars (“ n ”). It is reasonable that “ W_2 ” monotonically increases with pillar density as well as flow rate. However, our experimental results of higher flow rates deny this tendency (Fig. 2(c)). According to the data obtained, “ W_2 ” increases with the flow rate in a fashion of S-curve for most of pillar densities (Fig. 2(c)). For instance, “ W_2 ” for the cases of pillar interval from $300\ \mu\text{m}$ to $500\ \mu\text{m}$ reaches a plateau level as flow rate increases to $4500\ \mu\text{L h}^{-1}$ or above. It becomes worse for the densest case of 34 pillars, where a peak of “ W_2 ” occurs at $2500\ \mu\text{L h}^{-1}$, and then “ W_2 ” starts to fall with the increase of flow rates. The slight decrease of wake width at high flow rate, which is intuitively revealed by the gray value (analysing with ImageJ) varying with the dashed line in Figs. 2(b-2) and 2(b-3) from down to up (Fig. 2(d)), indicates that cells move towards the centerline at the sacrifice of the dim band nearby. It turns out that the previous speculation of monotonic increase in “ W_2 ” with pillar density as well as flow rate neglects an action on cells, which drives cells back, and this action may come from the channel walls. Like those cells flow over the long pillar (#7) as stated above, a cell-free layer is also well developed near the channel wall (Fig. S2(b) in the supplementary material⁵¹). With the accumulation of wall effect from both pillar surface and channel wall, deformable cells come closer resulting in interaction among them. As a result, the distribution of cells reaches to a new equilibrium reflecting “ W_2 ” reaches a plateau level and slightly goes down.

FIG. 2. Effect of pillar density in pillar array on F-L effect. (a) Schematic illustration of straight microchannel with pillar array. W_2 stands for width of wake induced by pillar array and Δx is the axial distance between the last pillar and the measured point, which is about $100\ \mu\text{m}$ in this study. The solution A is used for visualization. The pillar array is coded as No.- W_1 - n - ΔL , wherein “No.” refers to the type of pillar shape shown in Fig. 1, “ W_1 ” is the width of pillars in the array (about $80\ \mu\text{m}$ in this study), “ n ” and “ ΔL ” stand for the number and interval of pillars in the array, respectively. (b) The fluorescent images of wakes (corresponding to the dash box in (a)) in the microchannels with different pillar intervals at different flow rates. Scale bar is $100\ \mu\text{m}$. (c) Effect of the pillar density and the flow rate on the width of wake. (d) The gray value (analysing with ImageJ) varying with the dash line in Figs. 2(b-2) and 2(b-3) from down to up.

The plateau or falling segments of the S-curves manifest that the cells interact earlier than the last pillar. At extremely high flow rate, $9000 \mu\text{L h}^{-1}$, the both wall-effect lift forces from pillars and wall are so strong that “ W_2 ” has to compromise to a certain value. Moreover, the inertial effect of geometry-induced secondary flows^{37,38} on the “ W_2 ” should be also considered in channel with small interval at high flow rate.

One exception occurs in the case of the large pillar interval of $1000 \mu\text{m}$ (#1-80-7-1000) where “ W_2 ” monotonically increases with the flow rate from 200 to $9000 \mu\text{L h}^{-1}$. With the low frequency in lift force, the maximum width of wake for #1-80-7-1000 at the flow rate of $9000 \mu\text{L h}^{-1}$ is as half as those pillar arrays with small intervals. It seems that its critical flow rate for the peak in its S-curve is far above $9000 \mu\text{L h}^{-1}$. However, compared with the single pillar #7 (#7-80-1-0 in Fig. 2(c)), most of pillar arrays with pillar #1 enhances the width of wake significantly (Fig. 2(c)), although the single pillar #7 leads to much wider wake than single pillar #1. So the periodical frequent lift force plays a more significant effect than continuous lift force on cells, while the continuous one is distinct at low flow rate. The wake in microchannel with pillar array #1-80-34-200 is about 10 times wider than that of single pillar #1 at $5000 \mu\text{L h}^{-1}$. The microchannel with a pillar array especially that with high pillar density may further improve the robustness of H-filter for membraneless hemodialysis.

3. Effect of pillar diameter

Fig. 3 shows the effect of pillar diameter (W_1) in pillar array on the F-L effect. The pillar array with pillar interval of $500 \mu\text{m}$ (#1- W_1 -14-500) is used here. The fluorescent images of wake induced by pillar array with different diameters of pillars at the flow rates of $1000 \mu\text{L h}^{-1}$,

FIG. 3. Effect of pillar diameter in pillar array on F-L effect. (a) The fluorescent images of wakes induced by pillar array with different pillar diameters at different flow rates. Scale bar is $100 \mu\text{m}$. (b) Effect of pillar diameter and flow rate on the width of wake in the microchannels.

$5000 \mu\text{l h}^{-1}$, and $9000 \mu\text{l h}^{-1}$ are shown in Fig. 3(a). With the pillar diameter increasing from $60 \mu\text{m}$ to $100 \mu\text{m}$, the wake becomes wider at the constant flow rate. Fig. 3(b) displays the dependence of the wake width in the microchannel with different pillar diameters on the flow rate. The wake width increases faster with increasing the flow rate, and reaches a plateau level at high flow rate. The larger diameter as well as higher flow rate provides stronger lift force on cells and enhances the F-L effect, while the stable wake is due to wall-effect lift force as mentioned above. Therefore, the robustness of H-filter can be improved by increasing the diameter of pillar array either. However, considering the pillar array with large pillar diameter such as $100 \mu\text{m}$ will decrease the flowing area and thus be easy to clog, the pillar diameter of $80 \mu\text{m}$ is used to conduct the following experiments.

4. Effect of pillar number

Effect of pillar number of pillar array on the F-L effect is also investigated. Fig. 4(a) shows the fluorescent images of wake at different downstream regions in microchannels with different pillar intervals at a constant flow rate of $5000 \mu\text{l h}^{-1}$. The x axis indicates the axial distance away from the center of the first pillar. The wake develops faster and larger in the microchannels with smaller pillar interval. For the pillar array #1-80-34-200 and #1-80-22-300, the wake

FIG. 4. Effect of pillar numbers on F-L effect. (a) The fluorescent images of wakes at different downstream regions in microchannels with different pillar intervals at the constant flow rate of $5000 \mu\text{l h}^{-1}$. The x axis indicates the axial distance away from the center of first pillar. Scale bar is $100 \mu\text{m}$. (b) The dependence of wake width, which is measured at the each middle point of adjacent two pillars in the array, on the axial distance.

seems fully developed at $x = 3\text{--}3.4\text{ mm}$; while the wake is still developing at $x = 6.4\text{--}6.8\text{ mm}$ for other pillar arrays. The wake width measured at each middle point of two adjacent pillars in the array is plotted in Fig. 4(b) to see how the wake develops in the microchannel. It is clear that the wake width increases faster at the first dozens of pillars, and then goes up slowly. The smaller the pillar interval is, the shorter the axial distance is needed to reach the maximum wake width. It turns out that only a half of pillars in pillar array #1-80-34-200 is necessary to get the maximum value of wake as shown in Fig. 4(b). To develop the same width of wake, the less axial distance is needed for pillar array with smaller interval, which results in more compact microchannel. This result provides valuable guidance for the design of microchannels with pillars for highly efficient dialysis. Moreover, the *CP* cells (bright spots in Fig. 4(a)) adhere to pillars at distance between 0–0.5 mm; but nearly no *CP* cells appear on pillars at distance between 3–3.4 mm and 6.4–6.8 mm. This means the clogging and fouling decrease with distance increasing, which is due to the wider cell-free layer and the smoother streamline away from centerline in the downstream.⁴⁶ The result shows wider wake and smoother streamline in the microchannel will decrease the cell adhesion, which is also important to the following design of microchannels.

B. The robustness of enhanced microchannels

To further test the robustness of the wake in microchannel with pillar array, the enhanced microchannel with four-row pillar array as well as the microchannel with one-row pillar array and serial *S*-shaped channel are designed and fabricated. According to the above-mentioned results, the pillar array #1-80-34-200 with small pillar interval is used in the microchannel. In the microchannel with four-row pillar array (Fig. S3(a) in the supplementary material⁵¹), there are four wakes generated by each row of pillar array as shown in the fluorescent image (Fig. S3(b)⁵¹). All the four wakes are robust and flow without interference at flow rate of $15\,000\ \mu\text{l h}^{-1}$. The result also demonstrates the possibility of parallel dialysis by multiple-row pillar array in order to achieve the high throughput. Besides, the wake induced by the one-row pillar array always keeps robust as flowing through the serial *S*-shaped channel at the flow rate of $5000\ \mu\text{l h}^{-1}$ (Figs. S3(c) and S3(d)⁵¹). The results verify the robust wake in the enhanced microchannel, which is potential to further extend to the H-filter for robustness of dialysis.

C. Efficient mixing in enhanced H-filters

The high mixing efficiency of two fluids in the microchannel will lead to high efficiency of dialysis. The mixing efficiencies in H-filters with and without pillar array are investigated and compared. The H-filter without pillar array is called original H-filter (Fig. 5(a)), and that with pillar array is enhanced H-filter (Fig. 5(b)). The pillar array #1-80-34-200 is used in enhanced H-filter. Isotonic FeCl_3 solution (solution B in Figs. 5(a) and 5(b)) and KSCN solution (solution C in Figs. 5(a) and 5(b)) are pumped into H-filter at the same individual flow rate and used for visualization of chaotic mixing. The mixing process of two fluids at total flow rate of $5000\ \mu\text{l h}^{-1}$ along the H-filter is schematically illustrated in Figs. 5(a) and 5(b), and the optical images of the inlet, middle and outlet zones are also presented. The mixing of two fluids in the enhanced H-filter is more violent compared with that in the original H-filter.

The mixing efficiency (M) of two fluids in the H-filter is defined as the mixing percentage and calculated by the following equation:

$$M = (1 - \sigma) \times 100, \quad (1)$$

where σ refers to the standard deviation of gray intensity in a selected area, which is evaluated by calculating the deviation of the pixel intensity values I_i from the minimum intensity value in a selected area (Eq. (2)),⁴⁷

$$\sigma = \sqrt{\frac{1}{N} \sum_{i=1}^N (I_{\min} - I_i)^2}, \quad (2)$$

FIG. 5. Efficient mixing in enhanced H-filter. (a) and (b) Schematic illustration of the original H-filter (a) and the enhanced H-filter (b), and the optical images of the different zones in the H-filters (a-1)–(a-3) and (b-1)–(b-3). Isotonic FeCl_3 solution (solution B) and KSCN solution (solution C) at the same individual flow rate are used for visualization of chaotic mixing. Scale bars are $100\ \mu\text{m}$. (c) The dependence of the mixing efficiency (M) of two fluids in H-filters at total flow rate of 5000 and $9000\ \mu\text{L h}^{-1}$ on the axial distance. (d) The dependence of the mixing efficiency on the total flow rate and axial distance. The mixing efficiency is plotted by Origin utilizing TPS algorithm with the value of smoothing 1.

where N is the number of pixels in the selected area, I_i is gray intensity of pixel i in the selected area, $I_{\min}$ is the minimum gray intensity. The σ value of 1 and 0 denotes no and complete mixing, respectively. The mixing efficiency of two fluids in the original and enhanced H-filter at different zones varying with the total flow rate is analysed by MATLAB.

Fig. 5(c) shows the mixing efficiency values of two fluids at the total flow rate of $5000\ \mu\text{L h}^{-1}$ and $9000\ \mu\text{L h}^{-1}$ in the original and the enhanced H-filters vary with axial distance x . With the axial distance increasing, the mixing efficiency in the enhanced H-filter increases faster than that in the original H-filter, and reaches plateau level after the axial distance $x=5\ \text{mm}$. At the same total flow rate, the mixing efficiency in the enhanced H-filter is much higher than that in the original H-filter, which is in accordance with previous works.^{38–40} Meanwhile, with increasing the flow rate, the mixing efficiency in enhanced H-filter increases, indicating possible efficient dialysis at high throughput. However, the original one goes contrary. The higher mixing efficiency in the enhanced H-filter is due to the formation of secondary flow.³⁸ The dependence of the mixing efficiency in H-filter on the total flow rate and axial distance is further investigated and plotted by Origin utilizing Thin Plate Spline (TPS) algorithm with the value of smoothing 1 (Fig. 5(d)). With increasing the total flow rate, the mixing efficiency in the enhanced H-filter decreases fast first and then increases slowly, which leads to the minimum of the mixing efficiency. However, the mixing efficiency in the original H-filter monotonically decreases with increasing the total flow rate. At high flow rate with larger inertial effect, the mixing efficiency in the enhanced H-filter is always higher than that of the original filter along the axial distance. The mixing efficiency in enhanced H-filter is up to two times higher than in original one. The result verifies that the H-filter with pillar array improves the mixing efficiency of two fluids, which is expected to achieve highly efficient dialysis without membrane. According to the simulation guidance,³⁸ we can design more complicated pillar array to increase mixing on the premise of robust performance.

D. Dialysis without membrane

According to the results on optimization of microchannel, the single pillar #7 and pillar array #1-80-34-200 in the microchannel provide wide wake for robust dialysis and smooth streamline for decreasing cell adhesion and also long-time operation without clogging. Therefore, the H-filter with combining single pillar #7 and pillar array #1-80-34-200 is designed and fabricated for dialysis (Fig. 6(a)). First, the dialysis in such H-filter is tested by using FeCl_3 solution containing *CP* cells (i.e., solution D in Fig. 6(a)) and solution C at the same individual flow rate of $2500 \mu\text{l h}^{-1}$. Unfortunately, loss of *CP* cells is observed in the fluorescent images of Fig. 6(b) in the microchannel corresponding to the dash box in Fig. 6(a). *CP* cells may enter the dialysate fluid (solution C) at the arrow upstream as shown in Fig. 6(a), and flow along with the red dashed line and finally exit from the lower outlet. The reason is that the limited width of wake induced by single pillar #7 at the entrance of H-filter is not enough for robust dialysis if the disturbance occurs.

To improve the H-filter, a pillar array #1-80-17-200 is added between the single pillar #7 and pillar array #1-80-34-200, since half of the pillars (i.e., 17 pillars) in pillar array #1-80-34-200 are enough to get maximum wake according to Fig. 4(b). Moreover, the thin barriers with thickness of $20 \mu\text{m}$ (Fig. S4(a) in the supplementary material;⁵¹ “ W_3 ” in Fig. 6(c)) are used to connect two adjacent pillars of single pillar #7 and pillar array #1-80-17-200, which separate the two fluids before the wake reaches maximum upstream as shown in Fig. 6(c). The dialysis in the optimized H-filter is also tested by solutions D and C at the same individual flow rate of $2500 \mu\text{l h}^{-1}$ (Fig. S4(b);⁵¹ Fig. 6(d)). The optical and fluorescent images of the different zones in H-filter (corresponding to the dash box in Fig. 6(c)) are overlaid by ImageJ and shown in Fig. S4(b).⁵¹ To get clear distribution boundary of *CP* cells and $[\text{Fe}(\text{SCN})_n]^{(3-n)}$ ($n=1-6$), the overlaid image of dialysis (Fig. S4(b)⁵¹) is converted to grayscale image (Fig. 6(d)) with enhanced contrast by MATLAB. The blue dotted-dashed line stands for the centerline of microchannel (i.e., interface of two fluids), the red and yellow dashed lines represent for boundaries of *CP* cells and Fe^{3+} diffusion (mixing boundary). As shown in Fig. 6(d), no loss of cells is observed while the small molecules mix well. At the entrance of H-filter (Fig. 6(d-1); Fig. S4(b-1)⁵¹), the *CP* cells flow far away from the centerline of microchannel by the pillar-induced lift force. Cells flow steadily (Fig. 6(d-2); Fig. S4(b-2)⁵¹) and finally exit from the upper outlet without cell loss (Fig. 6(d-3); Fig. S4(b-3)⁵¹). Moreover, the blood red $[\text{Fe}(\text{SCN})_n]^{(3-n)}$ ($n=1-6$) is observed in the solution C in the middle and outlet of the H-filter (Figs. 6(d-2) and 6(d-3); Figs. S4(b-2) and S4(b-3)⁵¹). It verifies the small molecules (i.e., Fe^{3+} ions) can easily diffuse across the interface between two fluids and react with SCN^- . The results demonstrate that the optimized H-filter undergoes the robust and highly efficient dialysis and can be further used to hemodialysis.

For dialysis of diluted solution of red blood cells, equal flow rate of FeCl_2 solution containing blood cells (solution E) and $\text{C}_{12}\text{H}_8\text{N}_2$ solution (solution F) are used at total flow rate of $5000 \mu\text{l h}^{-1}$. Reynolds number is about 13.8 calculated based on water. At such low Reynolds number, blood cells are not injured for the low shear stress.⁴⁸ The images (Fig. S4(c) in the supplementary material⁵¹) of the different zones in H-filter during hemodialysis are also optimized by MATLAB, and the grayscale image is shown in Fig. 6(e). Just like the significant wake of blood diluted by NaCl solution induced by pillar array of #1-80-34-200 in straight microchannel (Fig. S4(d)⁵¹), the wake in H-filter (Figs. 6(e) and S4(c)⁵¹) at same total flow rate of $5000 \mu\text{l h}^{-1}$ is also significant. Significant wake guarantees no loss of blood cells during hemodialysis. Moreover, the diffusion boundary shows there is well mixing with small molecules. The blood cells and small molecules are separated at the outlets. Therefore, the optimized H-filter is practical for highly efficient and robust membraneless dialysis with the simplified blood for the first time.

The potential to large-scale parallel dialysis in optimized microchannel with five inlets and four-row pillar array is further investigated (Fig. S4(e) in the supplementary material;⁵¹ Fig. 6(f)). Each row of pillar array in microchannel is combined by a singled pillar #7, pillar array of #1-80-17-200 and pillar array of #1-80-34-200. The solutions D and C are alternately pumped into the

FIG. 6. Dialysis without membrane. (a) Schematic illustration of dialysis in H-filter with combining single pillar #7 and pillar array #1-80-34-200. FeCl_3 solution containing *CP* cells (solution D) and solution C are used for dialysis at the same individual flow rate of $2500 \mu\text{l h}^{-1}$. *CP* cells may enter the solution C at the arrow upstream, flow along with the red dash line and finally lose once the disturbance occurs. (b) The fluorescent images of dialysis at different zones in the H-filters (corresponding to the dash box in (a)). The loss of *CP* cells is observed. (c) Schematic illustration of dialysis in H-filter with combining single pillar #7, and pillar array #1-80-17-200 and #1-80-34-200. The thin barriers with thickness of $20 \mu\text{m}$ (W_3) are used to connect adjacent pillars of single pillar #7 and pillar array #1-80-17-200. The solution D and solution C are used for dialysis with cells, while the FeCl_2 two solution containing blood cells (solution E) and $\text{C}_{12}\text{H}_8\text{N}_2$ solution (solution F) are used for hemodialysis. Two fluids are pumped into the microchannel at the same individual flow rate of $2500 \mu\text{l h}^{-1}$. (d) The grayscale image of dialysis with *CP* cells. The images are optimized by MATLAB from the overlay images (Fig. S4(b) in the supplementary material⁵¹). (e) The grayscale images of hemodialysis (from Fig. S4(c)⁵¹). (f) The grayscale image of parallel dialysis with *CP* cells in microchannels with five inlets and four-row pillar array (from Fig. S4(e)⁵¹). Each row of pillar array in microchannel is combined by a single pillar #7, pillar array of #1-80-17-200 and pillar array of #1-80-34-200. The solutions D and C are alternately pumped into the five inlets at the same individual flow rate of $2500 \mu\text{l h}^{-1}$ (i.e., total flow rate of $12500 \mu\text{l h}^{-1}$). Scale bars are $100 \mu\text{m}$ in (b)–(e), and that is $200 \mu\text{m}$ in (f). The blue dotted dashed lines stand for the centerline of microchannel (i.e., interface of two fluids), and red and yellow dashed lines represent for boundaries of *CP* cells and mixing boundary (d)–(f).

five inlets at the same individual flow rate of $2500 \mu\text{l h}^{-1}$ (i.e., total flow rate of $12\,500 \mu\text{l h}^{-1}$) for parallel dialysis. The results are shown in Figs. S4(e) in the supplementary material⁵¹ and 6(f). The *CP* cells stay in the solution D and Fe^{3+} ions easily diffuses across the interface of two fluids along each row of pillar array. The streamline of fluids is smooth and stable downstream to the outlets. There is no loss of *CP* cells observed. Although the inlets are not precisely designed for equal pressure, the robust dialysis and reliable cell collection can still be obtained (Figs. 6(f) and S4(e)⁵¹). Moreover, the dialysate in parallel dialysis can be fully used for more efficient and economical outcomes.

Certainly, there will be a long way to realise membraneless hemodialysis with whole blood via the microfluidic channels in clinic. The complexity of whole blood and precise control of robust dialysis procedures are both the challenges. However, with broad concern and rapid advance of microfluidics, the “lab” is finally coming to the chip.¹⁶ We foresee the hemodialysis will also come to the chip in the future after the comprehensive understanding the hemorheology and developing the more practical microchannels based on advanced H-filter.

IV. CONCLUSIONS

In summary, an enhanced H-filter with pillar array based on F-L effect for efficient and robust dialysis without membrane has been successfully developed for the first time. We provide a simple way to quantify the F-L effect and optimize the structure of channel by utilizing the *CP* cells as blood model. The reliable collection of blood cells and chaotic mixing of small molecules are realized in the enhanced H-filter during the hemodialysis with the diluted solution of red blood cells. Meanwhile, it is demonstrated that the H-filter is potential to further extend for large-scale dialysis. The efficiency of dialysis could be further improved by modifying the enhanced H-filter into 3D microchannel with the advance of fabrication technology, such as confining cells inside ring array⁴⁹ or helical interfaces⁵⁰ in tubes to further increase the contact area of dialysis. Our anti-clogging design of H-filter provides possible guidance for complex dialysis, and our novel enhanced H-filter with pillar array based on F-L effect makes one step closer to wearable hemodialysis dialyzer.

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