Published in final edited form as:

Analyst. 2017 June 21; 142(12): 2220–2228. doi:10.1039/c7an00576h.

Micropatterned Macroporous Structures in Microfluidic Devices for Viral Separation from Whole Blood

Krissada Surawathanawises^a, Victoria Wiedorn^a, and Xuanhong Cheng^{a,†}

^aDepartment of Materials Science and Engineering/Bioengineering Program,, Lehigh University, Bethlehem, PA, 18015, USA

Abstract

Separation and enrichment of bio-nanoparticles from cell suspensions and blood are critical steps in many chemical and biomedical practices. We demonstrate here the design and fabrication of a microfluidic immunochromatographic device incorporating regular and multiscale monolithic structures to capture viruses from blood. The device contains micropatterned arrays of macroporous materials to perform size-exclusion and affinity chromatography in a simple flow-through process. The microscale gaps in the array allow the passage of cells while the macroporous matrices promote viral capture. Computational analyses reveal that fluid permeation into the porous matrices is controllable by the micropattern shape, separation distance and dimensions. Experimental analyses using blood samples containing human immunodeficiency viruses (HIV) as a model system further prove significantly improved viral capture efficiency using devices incorporating multiscale structures than those containing solid micropatterns. Such microfluidic devices with regular and multiscale structures have a potential for the separation and concentration of a wide range of bio-nanoparticles as well as macromolecules from complex mixtures containing both nano- and micro-sized species.

Graphical abstract

Microfluidic devices containing hierarchical features are designed to capture viruses from biological sample.

[†]Corresponding author: xuc207@lehigh.edu; phone: 610-758-2002.

Introduction

Separating and enriching bio-nanoparticles from a complex mixture of multiscale species are critical steps in many applications such as diagnosis of infectious diseases,¹⁻³ pharmaceutical productions of drug loaded vesicles,^{4, 5} and assessment of water and food safety.^{6, 7} Conventionally, bio-nanoparticle samples are processed through centrifugation,² filtration,⁵ electrophoresis,^{3, 8} magnetic separation,^{7, 9} and chromatography.¹⁰ Although these techniques have been routinely performed, complex reagents and procedures, sophisticated lab equipment, and skilled operators are often required. With rising demands of bio-nanoparticle processing and detection at resource-limited settings, alternative approaches for the separation and enrichment of these species are needed.

Microfluidic devices hold a great potential for sample processing with little infrastructure requirement. Miniaturized devices employing centrifugal,¹¹ magnetic,^{12, 13} filtration,¹⁴⁻¹⁶ chromatographic,¹⁷ and dielectrophoretic¹⁸ principles have been created to separate viruses and vesicles from cells. However, these devices face various challenges such as limited g-force to concentrate viruses, poorly controlled interactions between functional beads and target particles, clogging, and undesirable electrochemical reactions. Hydrodynamic fractionation based on inertial effect in microfluidic devices has been demonstrated to sort microparticles.^{19, 20} Yet the inertia effect is weak and less effective for nano-species.

Recently, immunoaffinity microfluidic devices have been reported to capture cells and pathogens in a simple flow-through process.^{21, 22} A high capture yield is warranted by matching the characteristic dimension of the capture bed with the target particles to promote their interactions. For example, solid micropost arrays have been introduced in microfluidic devices to isolate extremely rare circulating tumor cells from whole blood.²³⁻²⁵ Materials with nanoscale pores have been shown to isolate molecules and bio-nanoparticles with high yields.²⁶⁻²⁸ Considering a sample containing a mixture of both nano- and micro-sized species, such as viruses in blood, it has been hypothesized that a capture bed containing multiscale characteristic dimensions will outperform those with single scale micropatterns. To test this idea, Fachin *et al.*²⁹ fabricated an array of circular islands made of vertical carbon nanotubes, which allowed fluid to partially permeate into the porous micro-islands. Chen *et al.*³⁰ further utilized the device for cell capture and virus separation, and found that capture yields of both micro- and nanoparticles were enhanced compared to devices with solid islands. The enhancement is a result of reduced boundary layers around the islands, allowing close proximity of cells with the capture matrices and nanoparticle penetration. Yet, spacing of the carbon nanotubes is limited to 80 nm, which prevents the entry of larger nanoparticles, such as HIV virions.

Built on our prior work of macroporous material fabrication,³¹ we created micropatterned macroporous elements permeable to particles up to 200nm for HIV viral separation from human blood. We first investigated how the micropattern geometry and separation distance influence fluid permeation into the pattern using numerical simulations. Afterwards, we experimentally tested the capture of HIV particles from plasma and whole blood using one selected micropattern design. The results support the concept of enhancing particle separation by multiscale features in microdevices.

Experimental Sections

Computational fluid dynamics

2D simulations were carried out by COMSOL Multiphysics to evaluate fluid permeation into micropatterned macroporous islands. 2D micropatterns studied here included a circle, a half circle, and a half ring (Table 1). The patterns had porosity and permeability of 0.7 and $1 \times 10^{-13} \text{ m}^2$, respectively, corresponding to materials used in the experiments.³¹ Each pattern was centered between two parallel plates. The gap distance from the pattern edge to one plate wall was $D/2$. The Creeping Flow and Brinkman Equation modules were coupled in the simulation. The plates were set as periodic boundaries. An average inflow velocity of 1 mm/s was set at the inlet. The density and viscosity of the fluid were 1000 kg/m^3 and $0.001 \text{ Pa}\cdot\text{s}$, respectively. Volume percentage of fluid permeating into the porous element was calculated from Equation (1):

$$\text{Percentage of permeation} = \frac{\int_S (\mathbf{u} + \mathbf{v}) \cdot d\mathbf{s}}{\int_d (\mathbf{u} + \mathbf{v}) \cdot d\mathbf{x}} \times 100\% \quad (1)$$

where $\int_S (\mathbf{u} + \mathbf{v}) \cdot d\mathbf{s}$ and $\int_d (\mathbf{u} + \mathbf{v}) \cdot d\mathbf{x}$ are fluxes into the front arc of the porous elements and at the inlet, respectively. Dimensional variables studied here included radius of the patterns (R), gap distance between the pattern edge and the wall ($D/2$), and ring width (W , for the half-ring pattern only) (Table 1). Mesh convergence was confirmed first and the same mesh setting was applied to all three geometries.

Materials

SU-8 photoresist was obtained from MicroChem (Newton, MA). Sylgard 184 silicone elastomer kit was purchased from Dow Corning (Midland, MI). Polystyrene (PS) sheets were obtained from Plaskolite (Columbus, OH). A binary suspension containing 20 vol% of 1- μm silica beads and 8vol% of 100-nm PS beads was kindly provided by the Gilchrist lab at Lehigh University (Bethlehem, PA). Lyophilized bovine serum albumin (BSA) was obtained from Sigma Aldrich (St. Louis, MO). Phosphate buffered saline (PBS) was obtained from Mediatech (Herndon, VA). NeutrAvidin was purchased from Thermo Scientific (Rockford, IL). Green fluorescent 10- μm PS beads and red fluorescent 100-nm PS beads were purchased from Thermo Scientific (Fremont, CA). An HIV-1 p24 enzyme-linked immunosorbent assay (ELISA) kit was obtained from Perkin-Elmer (Waltham, MA). Human blood from healthy donors was kindly provided by the Office of Research and Innovation at Lehigh Valley Health Network (Allentown, PA).

Device fabrication

An array of half-rings was incorporated into microfluidic channels for HIV separation. The half-ring pattern had a height of 30 μm , outer radius (R) of 150 μm , gap distance (D) of 100 μm and ring width (W) of 50 μm . The half-rings were hexagonally arranged within an area of 25 mm $\times$ 10 mm (length $\times$ width). To fabricate the half-ring array, polydimethyl siloxane (PDMS) molds with a negative feature were created first using standard soft-lithography

techniques. The PDMS mold was treated by corona discharge to promote hydrophilicity. Next, a 10 μ L suspension containing 20 vol% of 1- μ m silica and 8 vol% of 100-nm PS was pipetted and spread on the PDMS mold (Figure 1(A)). The binary suspension was allowed to dry at room space. Then, the PS nanobeads were melted at 240 °C for 10 min to form a continuous matrix surrounding the silica beads. The sample was epoxied to a flat PS piece, and the PDMS mold was peeled off after the epoxy was cured (Figure 1(B)). At this point, solid half-ring patterns were revealed (Figure 1(C)). To create porous half rings, silica beads were removed by 50% hydrofluoric acid and the sample was rinsed by de-ionized (DI) water, leaving islands of PS networks of interconnected spherical pores (Figure 1(D)). To cap the device, a 25 mm $\times$ 8 mm PS roof drilled with an inlet and outlet was attached to the pattern array using double-sided tape (Figure 1(E)). The device was finally connected with tubing and sealed with epoxy glue. The final footprint of the micropatterned region was 25 mm $\times$ 8 mm as shown in Figure 1(F). temperature, and the silica beads self-assembled into a close-packed structure with the PS nanoparticles filling the interstitial space. Then, the PS nanobeads were melted at 240 °C for 10 min to form a continuous matrix surrounding the silica beads. The sample was epoxied to a flat PS piece, and the PDMS mold was peeled off after the epoxy was cured (Figure 1(B)). At this point, solid half-ring patterns were revealed (Figure 1(C)). To create porous half rings, silica beads were removed by 50% hydrofluoric acid and the sample was rinsed by de-ionized (DI) water, leaving islands of PS networks of interconnected spherical pores (Figure 1(D)). To cap the device, a 25 mm $\times$ 8 mm PS roof drilled with an inlet and outlet was attached to the pattern array using double-sided tape (Figure 1(E)). The device was finally connected with tubing and sealed with epoxy glue. The final footprint of the micropatterned region was 25 mm $\times$ 8 mm as shown in Figure 1(F).

The micropatterned macroporous structures were characterized by a field-emission scanning electron microscope (FE-SEM – Hitachi 4300) and the geometries were measured from SEM images using ImageJ software.

Surface modification

Microfluidic devices were functionalized with NeutrAvidin for specific capture of biotinylated HIV viruses. First, all devices were washed with 70% isopropanol and DI water. Afterwards, the microchannels were incubated with 10 μ g/mL NeutrAvidin for 2 hrs at room temperature to allow physisorption. Finally, the devices were rinsed with 1% BSA/PBS and used for viral capture immediately. To test nonspecific binding, devices were prepared by physisorption with 1 % BSA/PBS, instead of NeutrAvidin, for 2 hrs at room temperature.

Viral production

Pseudotyped HIV particles were cultured³² and biotinylated³³ following procedures from the literature. Briefly, HEK-293T cells were transfected with four plasmids of pGag-eGFP, pcRev, pR8 Env, and pJR-FL in DMEM using MegaTran 1.0. After a 24-48 hrs incubation period, the cells were washed with PBS and incubated with 1.67 mM Sulfo-NHS-LC-Biotin in PBS at room temperature for 30 min. The cells were then washed with PBS and 100 mM glycine to remove excess LC-Biotin, and re-cultured in the HEK culture medium for another 24 hrs. Subsequently, the supernatant was filtered and centrifuged to purify the biotinylated

HIV particles from cell debris. The labelling efficiency is $10^2 - 10^3$ biotin/virion. The viral sample was stored at -80°C until use.

Transport of fluorescent micro- and nanobeads in microfluidic devices

To visualize particle location in the micropatterned porous array, fluorescent micro- and nanobeads were used. A device was rinsed with 70% isopropanol and DI water, followed by incubation with 1% BSA/PBS for 2 hrs. Green fluorescent 10- μm PS beads and red fluorescent 100-nm PS beads were mixed together at equal volume and 0.5 vol% each. The suspension was injected into the device at 5 $\mu\text{L}/\text{min}$ for 60 min and then imaged. Afterward, the device was rinsed with PBS buffer at 20 $\mu\text{L}/\text{min}$ for 3 min and imaged for non-specific binding.

Viral capture in microfluidic devices

Biotinylated HIV was spiked into whole blood or plasma at a concentration of $\sim 10^6$ virions/mL. Then, the spiked blood or plasma was injected into the devices by a syringe pump at constant flow rates of 5, 20, and 100 $\mu\text{L}/\text{min}$ for a total volume of 300 μL . An iron ball was placed in the syringe of blood to manually stir blood periodically during the injection.³⁴ Afterwards, all devices were rinsed with PBS at 100 $\mu\text{L}/\text{min}$ for 5 min, and captured HIV was lysed on chip using 0.5% Triton X-100 flowing at 5 $\mu\text{L}/\text{min}$ for 10 min. Depending on the speed of virus flow, the sample processing time is between 18 - 75 min. The outflow from the viral injection, PBS wash, and Triton lysis steps were collected separately and analyzed for the amount of HIV virions. Volume of the PBS wash was tested ahead of time to ensure all free viruses were rinsed out before lysis. Flow-through samples containing blood were centrifuged to separate blood cells before HIV quantification. Amount of HIV was measured using a p24 ELISA kit. The input viral solution was also tested for HIV concentration to confirm mass balance between input and output. The capture results from 4 samples of each condition were statistically analyzed by a two-tailed Student's t-test.

Results and Discussion

Considering the application of bio-nanoparticle separation from cells, we hypothesize that an affinity capture bed containing hierarchical features, both on the micron and submicron scales, will outperform those with micro-features alone. To select an appropriate design to test this hypothesis, we first performed two dimensional COMSOL simulation to understand how geometry of porous micropatterns controls fluid permeation. We fixed the porosity and permeability, and varied the shape and spacing of the micropatterns. Using an average inlet velocity of 1 mm/s, dimensional analysis indicates that nano-particle transport in the microscale gap is controlled by convection mostly. On the other hand, nanoparticle transport inside the porous islands is diffusion dominated. Additionally, Chen et. al.³⁰ reported fluorescent molecules permeating gradually from the front to the back of a porous cylindrical post. These phenomena, combined with streamlines around a cylindrical island, suggest that the back of the micro-islands has lower probability to interact with penetrated nanoparticles for affinity separation, while removing the back could significantly reduce flow resistance and promote permeation. Thus, simulations were preformed for fluid

permeation in three different porous micropatterns, a full circle, a half-circle and half-ring to represent micro-islands of the corresponding x-y cross-sectional geometries. The pattern with the highest permeability and compatible with the fabrication process was then fabricated to test HIV capture from whole blood experimentally.

Computational analysis

We first used COMSOL Multiphysics software to simulate water permeation into macroporous patterns of three different geometries: a circle, a half circle, and a half ring, considering entrance from the front and plausible short mean-free path of nanoparticles in the porous affinity matrix. 2D geometries were placed between two parallel plates (Table 1). Three parameters were varied in the simulation: radius of the pattern (R), gap distance between the wall and the pattern edge ($D/2$), and ring width (W , for the half-ring pattern only). Figure 2(A)-(C) shows the profile of fluid velocity magnitude inside the three porous patterns with $R = 150\ \mu\text{m}$, $D/2 = 50\ \mu\text{m}$, and $W = 50\ \mu\text{m}$, and they are plotted on a comparable scale. As predicted, the velocity magnitude is the greatest at all locations along the front arc of the porous half ring, and lowest for the full circle (Figure 2(D)). Except for the poles, velocity magnitude is relatively uniform along the front arc of the half ring, but dips from the poles to the center in the other two geometries. The much-more-uniform velocity distribution along the front arc of the half ring geometry provides a more consistent environment for nanoparticle capture.

Percentage of water permeating into each porous structure was computed using Equation (1). We first investigated the effect of gap distance D varied from 50 to 200 μm with fixed $R = 150\ \mu\text{m}$ and $W = 100\ \mu\text{m}$ (Figure 3(A)). Permeation is at 1% or less in all simulated geometries, since the flow resistance of the macroporous patterns is much greater than that of the separation gap (D). At the same D , flux into the half ring is 4–9 times more than into the full circle, and flux into the half circle is in between. This is not surprising since flow resistance of a porous structure scales with its thickness, and the circular pattern is twice as thick as the half circle. The resistance is further reduced in the half ring of the same radius. Additionally, larger resistance of the circular pattern also makes it more sensitive to D . Permeation in the circle, half circle, and half ring drops by 22.9, 10.3, and 11.9 times, respectively, when D is widened from 50 μm to 200 μm .

Next we evaluated the effect of radius R in the range of 150 to 600 μm with fixed $D = 100\ \mu\text{m}$ and $W = 100\ \mu\text{m}$ (Figure 3(B)). The permeable fraction increases by 1.8, 1.3, and 3.5 folds with an increase of R from 150 nm to 600 nm for the circle, half circle and half ring patterns, respectively. Comparing the cases with variable D vs. variable R , the drop of the R/D ratio from 3 to 0.75 leads to one order of magnitude change in permeation with variable gap distance D , while the drop of the R/D ratio from 6 to 1.5 only produces a few times change in permeation with variable radius R . Thus D is a more effective factor to regulate fluid permeation into porous micropatterns. Qualitatively, this is understandable as the pattern radius generates two competing effects in controlling the flux: increasing the radius at a constant gap distance promotes flux by enhancing the path width of the porous matrices relative to the gaps, but the flow resistance through the porous matrices increases with radius to limit flux. The gap distance on the other hand, only changes the relative path width. It

should also be noted that the flow resistance in the three geometries don't scale linearly with the radius: the resistance around the poles are lower than around the equators. This is reflected as greater fluxes near the poles in Fig. 2(A)–(C), and causes nonlinear dependence of the flux on the geometrical parameters.

Furthermore, the effect of the width of the half-ring pattern W was studied. With all other parameters being fixed, $D = 100\ \mu\text{m}$ and $R = 150\ \mu\text{m}$, the permeable fraction increases as the width decreases (Figure 3(C)). Reducing the width from $100\ \mu\text{m}$ to $25\ \mu\text{m}$ leads to an improvement of permeation by 77%. This change of permeation is much slower than the change of the ring width, likely because permeation at the two poles are not significantly influenced by the ring width, while it contribute greatly to the overall permeation.

Porous cylindrical microposts have been reported to improve the capture yield of nanoparticles because fluid permeates into the structure and promotes nanoparticle transport into the capture bed.³⁰ Our simulation results suggest that micro-islands with a half-ring cross-section are more advantageous in promoting permeation, thus they are expected to further promote viral capture.

Fabrication of microfluidic channels with multiscale features

According to the simulation results, we fabricated microfluidic devices incorporating a monolith of hexagonally packed half-ring islands (Figure 4(A)). Both solid and porous arrays were created to compare the viral capture efficiency. The gap spacing, outer radius, and ring width are $100\ \mu\text{m}$, $150\ \mu\text{m}$, and $50\ \mu\text{m}$, respectively. Although narrower gap distance would promote greater permeation, as suggested by the simulation results, $100\ \mu\text{m}$ was selected to allow the passage of cell aggregates and avoid clogging. The ring width and radius were selected to provide enough mechanical stability for device assembly. The micropatterns were defined by a PDMS mold, into which a mixture of silica microbeads and polystyrene nanospheres was deposited followed with melting the polystyrene nanospheres (Figure 4(B)). After peeling off the PDMS mold, solid micro-islands were revealed. Porous micro-islands were created by further etching off silica beads (Figure 4(C)). The macroporous matrix presents an uniform spherical pore network with small interconnected neck of $\sim 233\ \text{nm}$ in diameter. The openings on the exterior surface have an average diameter of $696 \pm 56\ \text{nm}$. The pore density on the exterior surface is $\sim 1 \times 10^8\ \text{pores/cm}^2$ and the bulk permeability of the macroporous matrix is $\sim 1 \times 10^{-13}\ \text{m}^2$.³¹ The highly porous material allows permeation of fluids and transport of nanoparticles to the interior of the islands, which has a large surface area with affinity binding sites to capture the target species.

Transport of fluorescent micro- and nanobeads in the device

To demonstrate size exclusion of the porous micropatterns, $10\text{-}\mu\text{m}$ (green) and 100-nm (red) fluorescent beads were mixed together and flowed into a device with macroporous patterns. While most particles flow around the islands, a small number of $10\text{-}\mu\text{m}$ particles (green) slowly accumulate in front of the posts, while some 100-nm beads (red) are visible inside the porous posts (Figure 5(A)). The accumulation of microbeads is consistent with Chen's observation²⁴ that streamlines penetrate the islands. The nanobeads are carried into the islands through convection mainly and diffuse inside the islands before immobilization. Since

the device contains no affinity chemistry here, nonspecifically trapped microparticles can be mostly rinsed off by increasing the flow rate from 5 to 20 $\mu\text{m}/\text{min}$, while some nanoparticles remain inside the islands (Figure 5(B)). The observation of fluorescent particle distribution in the device confirms that the micropatterned porous device can separate the two different sized species.

Viral capture in micropatterned devices

Viral capture was performed first using virus spiked plasma (Figure 6(A)). The same volume of samples was flowed into functionalized devices at various flow rates of 5, 20 and 100 $\mu\text{L}/\text{min}$. For devices containing solid islands and functionalized with NeutrAvidin, the capture yield increases slightly from $1.04 \pm 0.14 \%$ to $2.29 \pm 0.38 \%$ when the flow rate drops from 100 to 5 $\mu\text{L}/\text{min}$. On the other hands, devices with the porous array and functionalized with NeutrAvidin yield significantly higher efficiency, ranging from $1.26 \pm 0.29 \%$ to $8.51 \pm 1.59 \%$ as the flow rate decreases from 100 to 5 $\mu\text{L}/\text{min}$. Nonspecific binding in the solid array devices treated with 1 % BSA remains relatively constant at $<1 \%$ regardless of the flow rate. In the BSA passivated porous array devices, the nonspecific binding increases from $0.48 \pm 0.22 \%$ at 100 $\mu\text{L}/\text{min}$ to $3.94 \pm 0.88 \%$ at 5 $\mu\text{L}/\text{min}$. The increased non-specific binding in porous/BSA devices over solid/BSA devices could be a result of some viral particles being physically trapped in the porous islands. Alternatively, BSA may not block non-specific binding well within the porous island under low shear, and it will be an interesting future study to characterize different surface passivation chemistries. Nonetheless, the difference in capture yields on BSA and NeutrAvidin treated materials suggests majority of viral particles are captured specifically.

Next viral capture was tested from whole blood using micropatterned devices. During blood injection, blood cells were found flowing around the patterned array and no clogging was observed. Similar to the capture results from plasma, higher capture yields are achieved at lower flow rates, while the yield is generally lower in blood than in plasma (Figure 6(B)). With NeutrAvidin-treated solid array, $0.89 \pm 0.14 \%$, $1.09 \pm 0.25 \%$, and $1.36 \pm 0.30 \%$ of virus is captured at the flow rates of 100, 20 and 5 $\mu\text{L}/\text{min}$, respectively. Functionalized porous array yields $1.15 \pm 0.38 \%$, $4.57 \pm 1.44 \%$, and $4.58 \pm 0.92 \%$ virus at these flow rates. Capture in BSA treated devices is lower than NeutrAvidin functionalized counterparts, while more nonspecific binding is observed in the porous array than the solid one. The greater nonspecific binding is a result of greater surface area in the porous array. At the same time, velocity is low inside the porous matrix, which leads to a weak wall shear force that may not insufficient to detach nonspecifically bound particles.

Compared to that in plasma, the capture yield in whole blood is reduced, suggesting insignificant margination of viruses in whole blood. Margination of white blood cells and platelets have been reported both in vivo and in microchannels, due to the preference of concave shaped and deformable red blood cells to travel in center stream.^{35, 36} However, margination of bio-nanoparticles remains a subject of debate. Toy *et al.*³⁷ demonstrated that liposomes sized 60-130 nm can marginate to microchannel walls. In contrast, Charoenphol *et al.*³⁸ reported that particles sized below 500 nm do not marginate. Lee *et al.*³⁵ also showed that 10-nm to 200-nm polystyrene beads are homogeneously dispersed under a capillary

flow both in vivo and by computational analyses. Our results seem support the latter view. Another possibility to lower yield from blood is the presence of marginated white blood cells and platelets as well as temporarily trapped cells at the front line of the islands, which impede the penetration of HIV particles into the porous micropatterns. Teasing apart these possibilities, however, will require additional investigation.

Overall, microfluidic channels containing multiscale materials enhance the capture yield of bio-nanoparticles from a complex fluid such as blood. The microscale gaps permit the passage of blood cells, while the macroporous structure inside the micropattern allows nanoparticle permeation for affinity capture. The Péclet numbers of viral particles in the microscale gap and macroporous matrix are on the order of 10^4 and 10^{-2} respectively. Thus different transport mechanisms dominate inside and out of the micropatterns. Even on the surface of the porous islands, velocity is non-zero. Using velocity values obtained from simulation on the surface of the half-ring island, the Péclet number of viral particles is still on the order of 10, suggesting convection carrying the particles into the islands. This contrasts with transport near solid islands, where velocity is zero on the boundary and nanoparticles need to diffuse through the boundary layer for affinity capture. Thus, porous micro-islands have two advantages for viral capture compared to solid micropatterns. First, the affinity area is improved by orders of magnitude with the macroporous structures. Second, the porous micropattern allows active transport of nanoparticles to the affinity surface. As a result, the porous array captures up to 4 times more HIV virions than the solid array. Similarly, Chen *et al.*³⁰ demonstrated that a porous array of intertwined carbon nanotubes enhanced capture efficiency of cells and bacteria by ~6 times on the exterior surface of the microposts compared to a solid array of the same geometry, due to a reduced boundary layer.

Although lab stocks of viral particles with biotinylated envelop are used in this study, the device is adaptable for antibody based viral capture. Since capturing bionanoparticles via antibody-antigen interactions have been reported under flow³⁹ and transport within the porous islands is diffusion dominant (Péclet number < 1), the weak shear within the porous islands is not expected to detach particles bound by antibodies instead of strong biotin-avidin interactions. In addition, prelabeling target particles by biotinylated antibodies could render biotin functionalities on the virus for direct capture with avidin treated devices.⁴⁰ The device fabrication process could also be adapted to the capture of other size particles through templating silica beads of appropriate sizes. While it is not studied here, it remains an interesting question how capture can be optimized by the structure of the macropores: larger pores could potentially promote more fluid permeation, but at the same time distance the target particles from the pore walls. Optimizing these two competing effects will be studied in our future work.

While permeation is not expected to change with the flow rate, the residence time is reduced as the flow rate increases. Short residence time limits viral diffusion from the central streamline in the interpost gap to the micropost surface and leads to dampened capture efficiency at high flow rates. The capture yield, although only up to 10% using a flow rate of 5 $\mu\text{l}/\text{min}$, is comparable to the separation yield of many flow-through plasma extraction devices reported in the literature.⁴¹⁻⁴³ Compared to those devices, our device offers

additional capability to enrich and purify target particles during continuous flow. In addition, microfluidic devices contain no macroporous structures have been reported in the literature for viral or exosome detection,⁴⁴⁻⁴⁶ and strategies that can improving the capture yield could enhance the detection sensitivity of these devices.

Different from prior work where circular microislands were designed, we selected a half-ring array to reduce the flow resistance of the porous island and promote permeation. Although this may sacrifice the functional surface area, the significant increase in permeation would be advantageous to promote more receptor-ligand interactions. For example, the capture area was reduced by 45% when using the ring geometry instead of full circles in the experiment, yet the permeation was increased by over 5 times. Although simulation predicts better permeation with even narrower half-ring widths, the porous array can collapse during device assembly or fluid injection if the width becomes too narrow. The selected geometry represents a balance between performance and practicality. Also, the capture yield is expected to further improve with the capture-bed length and residence time. The micropatterned devices are operated with a simple flow-through process, which can continuously handle a large fluid volume. As a result, target particles can be separated and concentrated to facilitate downstream detection.

Conclusions

Microfluidic devices incorporating half-ring shaped, functionalized hierarchical microarrays are fabricated in this work and shown to capture viruses more efficiently than devices with solid arrays. The enhancement is a result of viral permeation into the porous islands and specific interaction with the large affinity area. Viral separation and enrichment happen in a continuous-flow process relying on physical and biochemical interaction, while little infrastructure is required. As an easy-to-use device for multiscale separation, the device may find applications in pathogen sample processing in resource-limited settings.

Acknowledgments

This work was supported by the National Institutes of Health No. 1R21AI081638, National Science Foundation SNM-1120399 and PA Department of Health CURE Formula. Royal Thai Scholarship is acknowledged for the support of K.S. We thank Gilchrist lab for the binary suspension samples. We thank C. Multari for kindly preparing cultured HIV stocks. We also thank T. Friel, M.D. and C. Beitler for supplying the blood specimen.

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Figure 1. Schematics showing the fabrication process of microdevices containing half-ring micropatterns with solid or porous structures. (A) Deposition of a binary suspension of polystyrene (PS) and silica beads in a micro-patterned PDMS mold. (B) Transferring the micropatterned half-ring array to a flat substrate. The micropatterns contain either (C) solid structures without silica bead removal or (D) interconnected spherical pores after silica bead removal. (E) Capping the array with a roof to form a microfluidic device. (F) Photograph of an assembled microfluidic device containing an array of half-ring micropatterns used for viral separation.

Figure 2. Profile of velocity magnitudes inside (A) circle, (B) half-circle, and (C) half-ring patterns. Flow is from the left to the right. The profiles are plotted on the same color scale. (D) Velocity magnitude at different locations along the front arc of the three geometries.

Figure 3. Computational results showing the effect of (A) gap distance D , (B) outer radius R , and (C) width W of half ring on the percentage of fluid volume permeating into different porous designs.

Figure 4. SEM images showing (A) a half-ring micropatterned array ($D = 100 \mu\text{m}$, $R = 150 \mu\text{m}$, and $W = 50 \mu\text{m}$); zoomed-in images showing exterior surfaces of (B) a solid island where silica beads are surrounded by a PS matrix and (C) a porous island after silica bead removal. The openings are $695 \pm 56 \text{ nm}$ on the exterior wall. All SEM images were taken at $\sim 50^\circ$ to 55° tilted. Scale bars are $500 \mu\text{m}$ in (A) and $10 \mu\text{m}$ in (B) and (C).

Figure 5.

Fluorescence images showing distribution of physically trapped 10- μm (green) and 100-nm (red) particles in a device with micropatterned macroporous islands. (A) While most of the beads flow around the posts, a small number of microbeads accumulate in front of the islands whereas some 100-nm beads penetrate inside the porous matrix. The images were taken after flowing the mixture for 60 min at 5 $\mu\text{m}/\text{min}$. (B) While most of the 10- μm beads can be rinsed off at 20 $\mu\text{m}/\text{min}$, some 100-nm beads remain physically trapped inside the porous array after rinse, resulting in faint yet observable red dots in the islands.

Figure 6.

HIV capture efficiency in the solid and porous array devices under different flow rates from (A) blood plasma and (B) whole blood. The devices were functionalized by 1% BSA/PBS or NeutrAvidin (NT-avidin). The error bars represent standard deviation from 4 samples. Statistical analysis was performed using a two-tailed Student's t-test at a 95% confidence interval.

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Table 1
Design variables of micropattern geometry for testing permeation fraction

Micro patterns	Gap distance(D, nm)	Radius (R, nm)	Width of the half ring (W, nm)
Circle	50, 100, 150, 200	150, 300, 450, 600	-
Half circle	50, 100, 150, 200	150, 300, 450, 600	-
Half ring	50, 100, 150, 200	150, 300, 450, 600	25, 50, 75, 100