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Review Article

# Hydrophoresis – A Microfluidic Principle for Directed Particle Migration in Flow

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**Abstract** Despite the stereotype that secondary flow fields induced by surface grooves are effective for microfluidic mixing and increase the entropy of different fluid flows, many efforts have been made to utilize the grooves for particle separation and focusing, decreasing the entropy of particle distribution. As part of these efforts, hydrophoresis has been proposed to define deterministic particle trajectories in grooved microchannels. Due to the simple, clogging-free, and high-throughput characteristics, hydrophoresis has become increasingly promising for blood separation in clinical applications and sheathless particle focusing in flow cytometric applications. In this review, I introduce and summarize the basic physics, design parameters, design principles, and applications of hydrophoresis to improve the fundamental understanding of hydrophoresis and expand its use. I also discuss the challenges of hydrophoresis and forecast its future direction.

**Keywords: Hydrophoresis, Cell separation, Blood separation, Sheathless focusing, Microfluidics**

## Introduction

Rapid advances in the fields of cell-based diagnostics and therapeutics demand the development of high-performance cell separation technologies.[1-23] Given the recent progresses in detecting circulating tumor cells (CTCs) and using them as a biomarker for liquid biopsy,[8-10] the rapid isolation of the rare target cells from a heterogeneous cell population has become an essential part of cell-based diagnostic platforms.[1-7] In addition, the absence of a non-invasive, high-throughput method for isolating therapeutic immune cells from peripheral blood is a potential constraint for automating the manufacturing process of autologous immune cell products.[11-14] Cell separation technologies have a wide range of applications from basic research to clinical uses for diagnosis and therapy. However, conventional separation technologies, including centrifugation,[15,16] affinity chromatography,[17,18] membrane filtration,[19] fluorescence-activated cell sorting (FACS),[20,21] and magnetic-activated cell sorting (MACS),[22,23] are limited by the requirements of time-consuming, complicated separation procedures, expensive equipment, or target cell modification with biochemical labels.

Reducing the dimensions of cell separation to microscales offers significant advantages over conventional macroscale systems.[24-81] For example, rapid and precise cell separation can be achieved by placing cells constrained in the thin layer of a microchannel closer to a separation medium (e.g., external force fields) and thus applying more uniform and stronger fields to the cells. In addition, the use of microchannels can reduce sample volume and power consumption for cell separation, thereby simplifying the whole separation system and enabling cost-effective separation. In the early days of microfluidic cell separation, researchers focused primarily on combining conventional separation principles with microchannels.[24-35] Electric, magnetic, optical, and acoustic fields have been integrated into microfluidic devices and proven their efficacy to resolve subtle differences in dielectric prop-

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erties,[25,26] magnetic susceptibility,[27-29] refractive index,[30,31] and acoustic compressibility,[32,33] respectively. Membrane filters or microfluidic sieve structures have been also integrated into microfluidic devices for straightforward cell separation based on size and deformability.[34,35] However, these methods typically require the substantial dilution of clinical samples with high cell concentrations (e.g., blood), since filter structures can be clogged and the response of cells to the separation fields can be disturbed by high cell concentrations. This is a common issue affecting most microfluidic separators that can result in low separation throughput and limit their clinical applications to process a large volume of samples.

New understandings in microfluidic physical phenomena, which are based on interactions between fluid flows and cells at micrometer scales, have led to great improvement in the performance of microfluidic separation in terms of resolution and throughput.[36-53] A seminar work by Sturm et al.[36] demonstrated high-resolution particle separation at micro- and nano-scales, showing great potential of precise particle alignment against microstructures for size-based bioparticle separation. The alignment angles of particles flowing through a micropillar array can be finely tuned by particle size and geometric parameters defining the array.[36-40] Similar steric effects have been implemented in simpler separation platforms:[41-44] for example, hydrodynamic focusing channels have been used to push particles against a channel wall, thereby precisely aligning them according their size.[41-43] Microfluidic channel networks for hydrodynamic filtration have been elaborately designed to drain excess fluid through side-channel branches and precisely align particles without the aid of sheath fluids.[44] Unlike these steric effect-based separators, inertial microfluidic separation typically does not require complex separation medium (e.g., post arrays and channel networks) and sophisticated particle control with sheath fluids.[45-53] Separation is enabled by inertial lift forces acting on particles in relatively high Reynolds number (Re) regimes ($1 < Re < 100$), thereby allowing high-throughput separation and changing the notion of separation throughput limitation in microfluidics. Exploiting inertial fluid dynamics at microscales results in a much higher separation throughput than other microfluidic principles and enables effective processing of a clinically-relevant large volume of samples.

In parallel to the development of the microfluidic separators, interactions between microfluidic groove patterns, groove-induced rotational flow fields, and bioparticles have been studied and used for particle separation and focusing.[54-81] These microfluidic phenomena were termed as hydrophoresis. The hydrophoresis separation mechanism is simple. Bioparticles differing in size, shape, and/or other physical properties are driven by groove-induced secondary flow fields into different lateral positions across a microchannel (Figure 1). In the secondary flow fields, the relative distributions of the bioparticles across the channel are determined by their physical interactions with groove patterns. The advantage of hydrophoresis is that clinical samples with high cell concentrations can be processed without dilution and sheath fluid control at a high throughput (up to 1 mL blood per min).[73-77,80] Despite their significant contributions to the field of microfluidic separation and focusing, there is no review article which introduces the fundamental physics of hydrophoresis and its applications in biomedical fields. In this review, I discuss the basic principles behind the microfluidic phenomena of hydrophoresis and their applications for blood separation and sheathless focusing. I also discuss the advantages and disadvantages of hydrophoresis, and provide my perspective on its future direction.

## Particle Migration Dynamics in Grooved Microchannels

### Microstructure-induced Secondary Flow

Hydrophoresis originated from the idea of using microfluidic chaotic mixing[82-88] to move particles in the lateral direction even in low Re regimes ($Re < 1$) where viscous forces become dominant compared to inertial forces. The first description of the advective flow phenomena at microscales termed as chaotic mixing was by Whitesides et al.[82] Their experiments using a grooved microchannel with an oblique angle found that fluid flows can be directed through surface grooves patterned on one side of the microchannel, and transverse pressure gradients induced by the guided flows generate uniform flow circulation or rotation through the channel (Figure 1). This surface pattern-directed flow control mechanism has been studied numerically for optimal mixer designs[85-88] and extensively used in various microfluidic applications: for example, controlled synthesis of lipid nanoparticles has been achieved by rapidly mixing lipid and aqueous solutions with periodically-varying groove patterns and complicated downstream fluid streamlines,[89,90] and efficient cell capture on antibody-coated surfaces has been demonstrated by forced cell contact on the surfaces.[91-93]

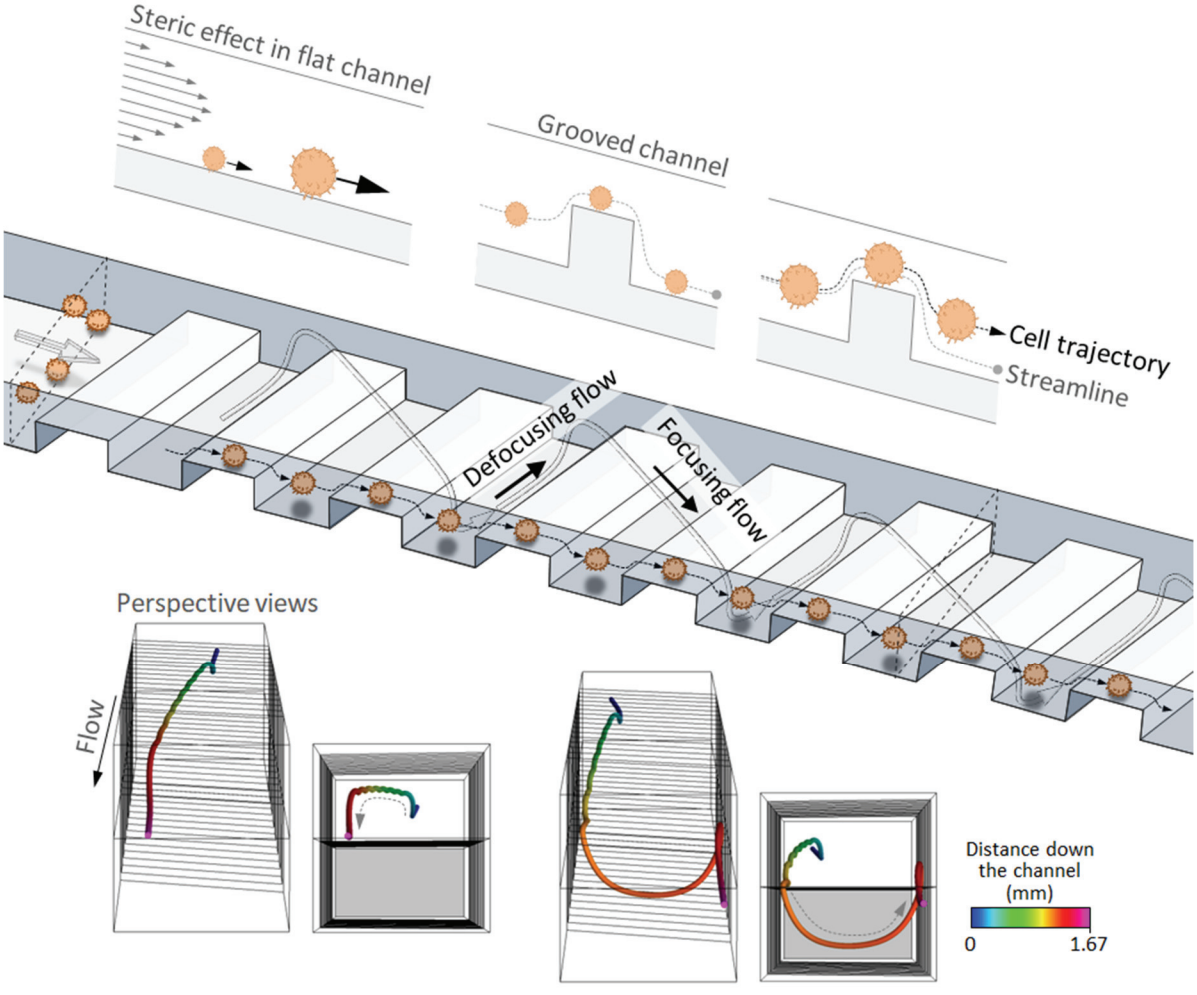


**Figure 1.** Schematic of directed particle migration by hydrophoresis. (Top) The vertical particle displacement by steric effects depends on particle size. (Middle) Deterministic particle trajectories in the grooved channel. (Bottom) Simulated streamlines for the rotational flow in the grooved channel.

## Steric Interactions Between Surface Grooves and Particles

The discovery of directed particle migration in grooved microchannels was accomplished by Park and me with numerous attempts to understand interactions between microfluidic groove patterns, groove- induced rotational flow fields, and bioparticles.[54] In a grooved channel with an oblique angle, particles are driven by groove-induced rotational flow fields, moving back and forth across the channel (Figure 1). Such particle motion can be constrained by the channel structure as the channel dimensions are reduced to a size similar to the particles. The overall flow fields are composed mainly of two currents flowing in opposite directions. Inside the grooves, the flow, termed as defocusing flow, is guided along the groove pattern, while above the groove surface, the flow, termed as focusing flow, circulates in the opposite direction (see the middle of Figure 1). In the rotational flow fields, particle distributions can be determined by which of the two flows particles are more affected. Larger particles can be distributed farther away from the groove surface by steric effects and thus they can focus to the sidewall following the focusing flow rather than the defocusing flow, showing a narrow particle distribution (see the top of Figure 1 and Figure 2a).[58,62,63] On the other hand, small particles whose physical interaction with the grooves are negligible can be affected by both flows and be broadly distributed across the channel. Thus, the degree of particle focusing by hydrophoresis depends highly on particle size. Hydro phoresis is defined as the directed migration and separation of particles under the influence of micro

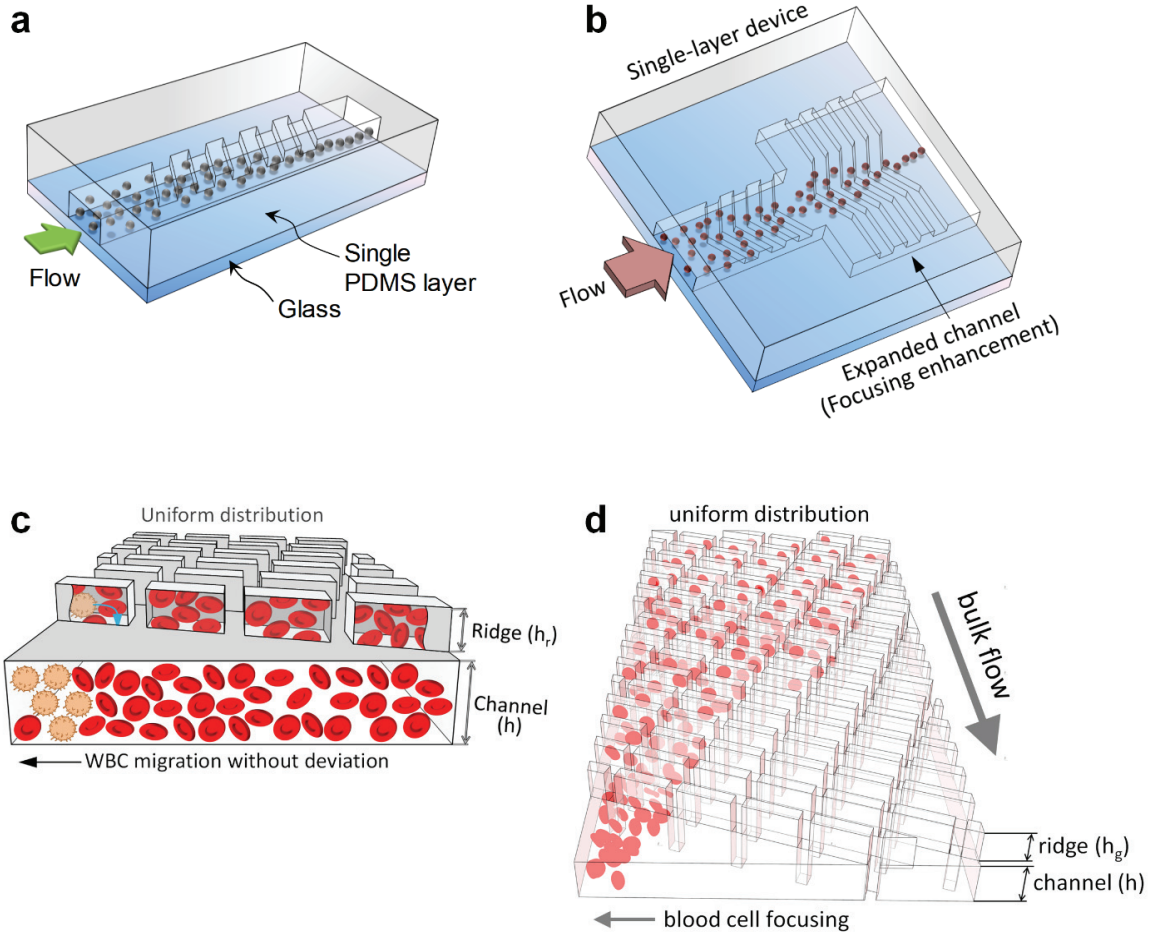


**Figure 2.** Groove designs for hydrophoresis. (a) Schematic illustration showing a continuous groove design and the resulting deterministic particle migration. Reprinted from ref. 59 with permission from the American Chemical Society. (b) A continuous Λ-shaped groove design with side extensions to improve focusing efficiency. Reprinted from ref. 66 with permission from John Wiley and Sons. (c) A discontinuous groove design to prevent WBC deviation along grooves and ensure robust WBC focusing. Reprinted from ref. 73 with permission from John Wiley and Sons. (d) A discontinuous groove design for robust focusing of all blood cell types and thereby blood plasma separation. Reprinted from ref. 81 with permission from the Royal Society of Chemistry.

structure-induced flow fields and steric interactions between particles and surface grooves. I note that hydrophoresis is similar to steric field-flow fractionation (FFF)[94] in that both techniques use steric effects for particle separation. Hydrophoresis provides significant advantages over conventional steric FFF techniques. The use of surface grooves allows the conversion of vertical particle distributions in steric FFF to horizontal distributions in hydrophoresis, thus enabling continuous separation and easy particle fractionation.

## Design Parameters for Hydrophoresis

Key design parameters for hydrophoresis include channel dimensions, particle size, groove patterns, and flow rate.[54,59,65]

### Groove and Channel Height

Groove height ($H_g$) determines the magnitude of microstructure-induced lateral pressure gradients ($P_L$). Park et al. demonstrated that $P_L$ increases with $H_g$ and saturates from the moment $H_g$ equals $H_c$ by numerical simulation,[59] where $H_c$ is the channel height except the groove region. They also experimentally verified that particle migration by hydrophoresis is significantly suppressed when $H_g/H_c$ was reduced to 0.1.[59] Thus, the ratio of $H_g/H_c$ should be larger than the unity for sufficient generation of $P_L$ and effective particle migration by hydrophoresis. At $H_g/H_c \geq 1$,

hydrophoresis is then governed by steric interactions between particles and surface grooves. Park et al. had experimentally and numerically demonstrated that steric effects become dominant when $H_c$ is reduced to less than twice $D_p$,[59,62] where $D_p$ is the particle diameter. Interestingly, hydrophoresis is valid in a wide range of $H_c$ from 1.2 μm to 20 μm, thereby allowing size-based separation of various bioparticles including cells and DNA molecules.[59,60,64,69-81]

### Channel Width and Groove Pattern

Channel width and groove pattern are key parameters which can change microstructure-induced secondary flow fields and thus affect particle distribution by hydrophoresis. As a grooved channel widens laterally, the corresponding secondary flow fields also spread across the channel.[65] The spread secondary flow fields can contain multiple localized rotational flow fields and particles can interact mainly with a certain local rotational flow field located near their equilibrium position.[65] Thus, the wider the channel width ($W_c$), the narrower the distribution of particles relative to the channel width. At $H_g$ = 23.5 μm, $W_c$ = 50 μm was an optimal condition for size-based particle separation, and for particle focusing, $W_c \geq 400$ μm.[65] Groove patterns also determine the direction of the focusing flow: the inclined direction of grooves is the same as the direction of the defocusing flow and the direction of the focusing flow is determined in the opposite direction. Thus, different groove patterns can produce different particle focusing patterns. For example, herringbone patterns rotated 45 degrees enable multiplexed particle focusing.[59]

### Flow Rate

As the flow rate ($Q$) increases in a grooved channel, not only hydrophroesis but also inertial forces begin to influence the equilibrium particle positions. The aspect ratio of the microchannels for hydrophoresis is usually far from the unity, in which case particles can focus to the center of the long channel walls by inertial forces.[45-48] If the equilibrium positions due to hydrophoresis and inertial forces are different, the resulting particle focusing positions will be unstable depending on $Q$ because the positions will be determined by hydrophoresis at low $Q$ (Re $\leq$ 1) and by inertial forces at high $Q$ (Re $\geq$ 20).[68] Such instability could be applied for a multifunctional channel that can perform different functions depending on $Q$, shifting the function of the channel from size-based separation by hydrophoresis to sheathless focusing by inertial forces as increasing $Q$.[68] If the equilibrium positions due to hydrophoresis and inertial forces coincide, robust particle focusing could be achieved regardless of $Q$, enabling particle focusing by hydrophoresis at low Re and inertial forces at high Re.[69] Optimum flow conditions for hydrophoresis vary depending on channel dimensions and applications, and thus have been experimentally found at each hydrophoresis device. A general rule is to run hydrophroesis devices at Re $\leq$ 1 for particle control only by hydrophoresis.

## Design Principles for Hydrophoretic Separators and Focusers

### Continuous Groove

A general groove design for hydrophoresis is a groove with an oblique angle while continuing across a channel without discontinuity,[54-72] termed as continuous groove (Figure 2a). The continuous groove has been proven to be effective for size-based separation of microparticles and cells:[54,59,60] for example, at $H_g$ = 23 μm and $W_c$ = 100 μm, polystyrene particles of 8, 9, 10, 11, and 12 μm nominal diameter were spatially resolved with different equilibrium positions across half the channel width. The continuous groove also allowed autonomous cell separation by cell size and cycle.[60] However, such size-based separation ability is unsuitable for hydrodynamic focusing applications that require aligning particles or cells into a narrow stream regardless of their size difference. To extend the applicability of hydrophoresis to sheathless focusing, grooved microchannels have been developed that were patterned with Λ-shaped grooves only in a portion of wide channels and guided particles of different size through the patterned grooves (Figure 2b).[57,66] Such channel design can lower the ratio of the particle distribution width to the channel width, while improving focusing efficiency.

### Discontinuous Groove

Although the continuous groove is effective for size-based particle separation, when separating clinical samples with high cell concentrations such as blood, cell-to-cell interactions become dominant, which can impair cell separation efficiency.[73-81] The major cause of reducing cell separation efficiency is that physical interactions between cells force some of them to enter grooves, move along the defocusing flow, and leave a focused cell stream. Groove designs with discontinuity have been developed to prevent cells from deviating from a focusing stream, and termed as dis-

continuous groove (Figures 2c and 2d).[73-81] The discontinuous groove designs have a structure in which numerous small grooves are arranged at regular intervals on a wide microchannel. These grooves could localize rotational flows within the narrow width of the grooves, thereby limiting cell deviation from a focusing stream within the narrow width and significantly improving cell focusing or separation efficiency. When foreign substances such as blood clots or fine dusts are trapped in microchannels, they can disrupt cell separation streams and impair separation efficiency. The discontinuous groove designs enabled robust separation by limiting the effects of the substances trapped in microchannels into the narrow width of the grooves and rapidly recovering a focusing stream.

### Combination of Hydrophoresis with Other Separation Principles

Hydrophoresis relies mainly on size differences for separation which can limit its applicability to the separation of particles with other physical-property differences other than their size. To address this issue, several efforts have been devoted to combining external force fields (e.g., electric or magnetic fields) with hydrophoresis (Figure 3).[95-98] Li et al. have shown that particle distributions in grooved channels can be controlled by applying magnetic or dielectrophoresis forces, and demonstrated that particles with difference in magnetic or dielectric properties can be effectively focused or separated in the grooved channels.[95-98] The use of grooved channels allows for easy fractionation of particles by converting vertical particle distributions by external force fields to lateral particle distributions. For example, particles drawn into grooves by external force fields can be sorted along the defocusing flow, while particles not affected by the fields along the focusing flow. As $Q$ increases, the equilibrium positions of particles can be greatly influenced by inertial effects. Therefore, it is necessary to optimize $Q$ for particle focusing or sorting by considering the relative effects of external fields and inertial forces.

## Applications of Hydrophoretic Particle Migration

### Sheathless Focusing

Hydrodynamic focusing is an essential technique for flow cytometry that can align cells to an interrogation point through sheath fluid control. The use of sheath fluids requires large-volume sheath and waste tanks, and complex fluidic control.[99,100] Sheathless focusing

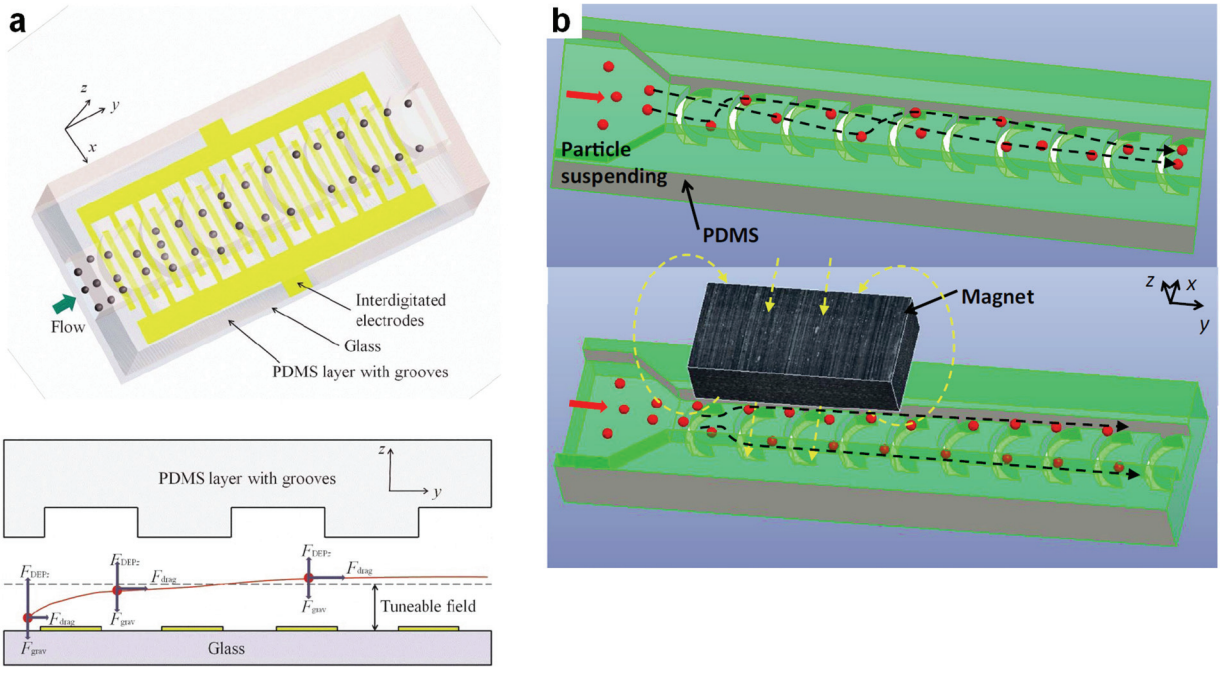


**Figure 3.** Microfluidic principles that combine hydrophoresis with (a) dielectrophoresis or (b) magnetophoresis. Reprinted from ref. 96 and ref. 98 with permission from Springer Nature, respectively.

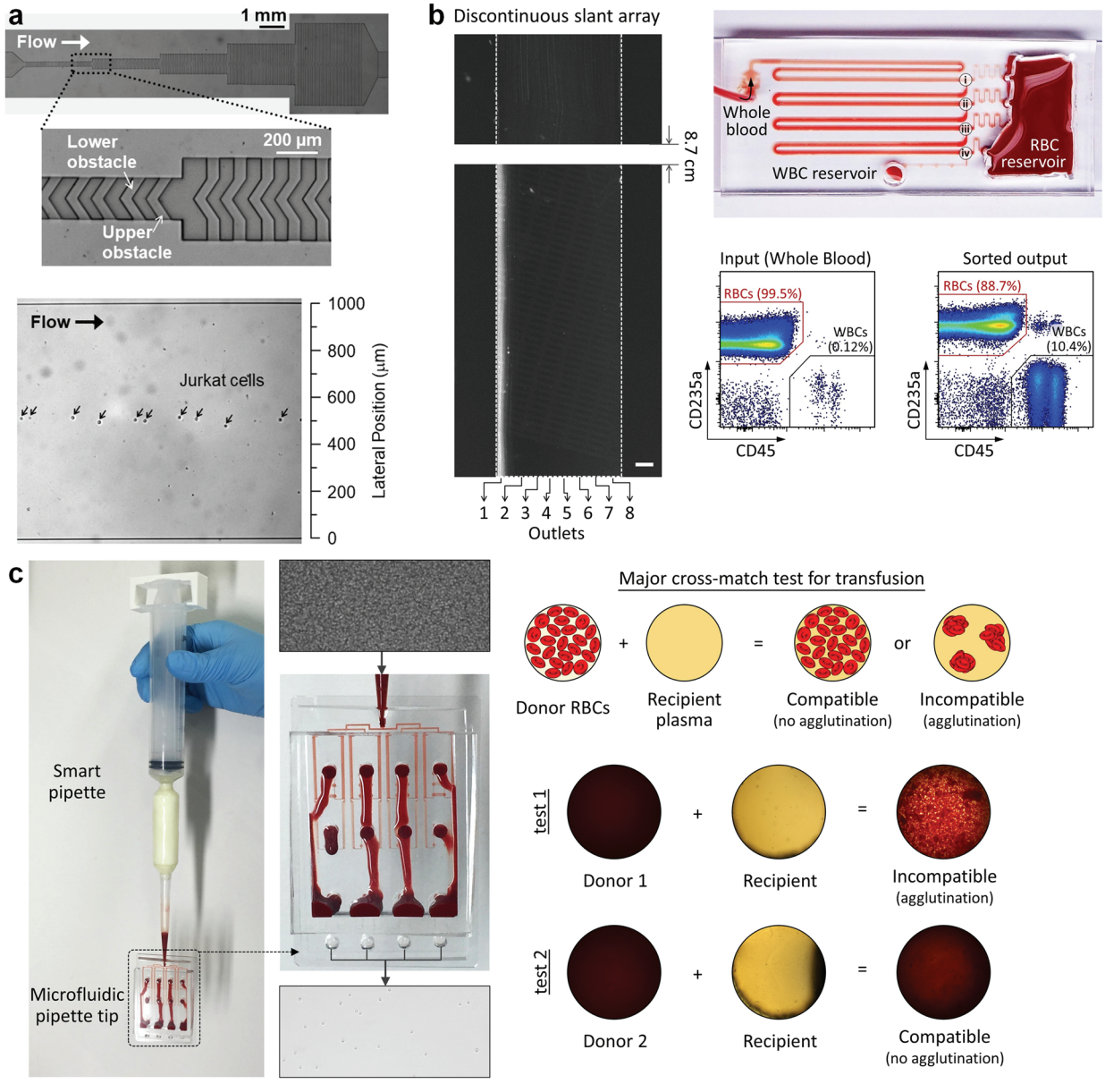


**Figure 4.** Applications of hydrophoresis. (a) Sheathless cell focusing by the continuous Λ-shaped groove design with side extensions. Reprinted from ref. 57 with permission from the American Chemical Society. (b) High-throughput WBC separation by the discontinuous groove design. Reprinted from ref. 73 with permission from John Wiley and Sons. (c) High-throughput blood plasma separation by the discontinuous groove design and its application to blood cross-match testing. Reprinted from ref. 74 with permission from the American Chemical Society.

is a promising technique that can obviate the need for sheath fluid control and simplify the fluidic system of flow cytometry, thereby enabling its miniaturization.[101-103] Directed particle migration by hydrophoresis has been utilized to focus particles to a desired position inside a microchannel (i.e., channel center) without the aid of sheath fluid control.[57,66,68,81] However, the dependence of the equilibrium particle positions on their size can cause significant variation in flow cytometry analysis by particle size. To address this issue, two approaches have been proposed; one of which was to guide particles of different size using locally-patterned grooves over a wide microchannel as described in the section 4.1. (Figures 2b and 4a),[57,66,81] and the other of which was to combine hydrophoresis and inertial effects as described in the section 3.3.[68] Both approaches enabled generation of a narrow cell stream (less than 5.6% in the coefficient

of variance (CV)).[66,68] The first approach was successfully applied to implement a portable fluorescent particle counter.[81] However, in order for both approaches to be used more versatilely, the first and second methods should overcome the particle-size dependency and the flow-rate dependency, respectively.

### Blood Separation

Blood separation is a technique to isolate target blood components and thereby prevent interference from non-target components, and is essential for therapeutic and diagnostic purposes. For example, T lymphocytes should be sorted from whole blood for immune cell therapy,[11-14] and blood plasma needs to be sorted from blood cells which can interfere with liquid biopsy assays.[104,105] A technical difficulty in blood separation is that numerous blood cells and their interactions can complicate microfluidic separation processes, thereby impairing separation efficiency. The discontinuous groove designs as described in the section 4.2. have been developed to minimize the effects of numerous blood cells and maintain the focusing stream of target cells without deviation.[73-81] Interestingly, white blood cell (WBC) focusing using a discontinuous groove design was maintained at a high flow rate of 125 μL/min (Figure 4b),[73] and WBCs were effectively isolated from whole blood even at a high processing rate of 1 mL/min through channel parallelization.[75] Recently, a significant improvement of WBC separation purity (≈97.5%) was achieved by synergistically combining hydrophoretic blood separation and microfluidic lattice-based cell washing techniques.[80] In addition, the discontinuous groove was successfully applied to high-throughput blood plasma separation by discarding blood cells focused by hydrophoresis and recovering the remaining plasma (Figure 4c).[74,76]

## Summary and Outlook

Hydrophoresis is a simple but effective technique for cell separation and focusing, and have demonstrated its versatility applicable to various bioparticles such as blood cells, DNA molecules, and nanoparticles. However, beyond principle verification and preliminary device testing, key challenges still remain for hydrophoresis to be practical in clinical use and translated into commercial products. Many microfluidic separation principles as well as hydrophoresis separate cells based on their physical differences such as cell size or deformability, while clinical separation typically requires cell separation based on immunophenotype. Thus, to extend the clinical applicability of hydrophoresis, technology development should be made to separate cells by immunophenotype as like commercialized MACS and FACS. Another challenge is to change the device fabrication material into a cheap, robust and mass producible material (e.g., polystyrene) rather than a prototyping material such as PDMS. In-depth studies for fabrication and quality control methods should be performed according to fabrication material change for further commercialization of hydrophoresis devices. By addressing these challenges, hydrophoresis will become a representative cell separation and focusing technique that will be favored by many academic, industrial and clinical users in the future.

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