Separation of sperm and epithelial cells based on the hydrodynamic effect for forensic analysis

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In sexual assault cases, forensic samples are a mixture of sperm from the perpetrator and epithelial cells from the victim. To obtain an independent short tandem repeat (STR) profile of the perpetrator, sperm cells must be separated from the mixture of cells. However, the current method used in crime laboratories, namely, differential extraction, is a time-consuming and labor-intensive process. To achieve a rapid and automated sample pretreatment process, we fabricated a microdevice for hydrodynamic and size-based separation of sperm and epithelial cells. When cells in suspension were introduced into the device's microfluidic channels, they were forced to flow along different streamlines and into different outlets due to their different diameters. With the proposed microdevice, sperm can be separated within a short period of time (0.5 h for a 50- μ l mock sample). The STR profiles of the products in the sperm outlet reservoir demonstrated that a highly purified male DNA fraction could be obtained (94.0% male fraction). This microdevice is of low-cost and can be easily integrated with other subsequent analysis units, providing great potential in the process of analyzing sexual assault evidence as well as in other areas requiring cell sorting. © 2015 AIP Publishing LLC.

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I. INTRODUCTION

The recovered genetic material in sexual assault cases is a mixture of epithelial cells (EPs) and sperm obtained from victims' vaginal swabs or undergarments. To obtain an independent short tandem repeat (STR) profile of the perpetrator, sperm cells must be separated from EPs. The conventional separation method, which is termed differential extraction (DE), utilizes the different stabilities of the membranes of the two cell types.^{1,2} Although DE is a well-established and robust separation method, it requires >3 h to obtain the purified sperm cells. Additionally, as DE includes multiple steps, such as cell lysis, centrifugation, and supernatant removal, it is labor-intensive and difficult to automate.

Various techniques have recently been employed by scientists to improve this separation procedure. In 2005, Horsman *et al.* separated sperm and EPs based on their different sediment

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velocities and different adherence properties. However, most of the sperm cells (>75%) remained in the inlet and were not recovered.³ In 2009, Norris *et al.* demonstrated the separation of sperm from EP lysate using acoustic differential extraction. This method relies on size-based acoustic trapping of sperm cells in the presence of EP lysate.^{4,5} Although the purity of the product was high, the devices were complicated and difficult to fabricate. Techniques such as filtration⁶ and dielectrophoresis (DEP)⁷ have also been used for separation, but the purity (<80% male fraction) of the product is low. Other techniques, such as laser microdissection^{8,9} and antibody-based fluorescence-activated cell sorting (FACS),¹⁰ have been reported as well. These methods are suitable for samples with low sperm concentrations, but their costs are relatively high.

For cell separation in forensic areas, the most important demand is the purity of the product. Disposable tools are widely used to avoid cross-contamination, and low cost is always a benefit. As a result, considering the increasing number of rape cases, a rapid, cost-effective, and easily automated method ensuring a highly pure sample is in high demand.

Microfluidic devices have been increasingly used in cell separation areas due to their advantages in precise handling of small particles and their potential for automation. Over the last two decades, there have been several attempts to fabricate microfluidic devices for cell separation. Methods utilizing extra fields, such as DEP fields,^{11,12} magnetic fields,^{13–15} and sound pressure fields,^{4,16} have achieved numerous good results. However, such methods require complicated microfabrication processes to generate these external force fields, leading to a high cost for the devices and complicated manipulation processes.

The use of the hydrodynamic effect offers an efficient and effective approach for cell separation, and the fabrication process for such microchannels is simple and mature. For relatively high Reynolds numbers ($Re > 20$), several microdevices have been reported for cell separation using the inertial force at a high flow rate.^{17,18} The high-throughput of these devices is superior, but as the membrane of EPs are relatively weak compared to other cells, high flow rates may lyse the EPs, resulting in free female DNA in the sperm fraction. For low Reynolds numbers ($Re < 20$), Yamada *et al.* (2004) proposed a method known as “pinched flow fractionation” to achieve size separation of particles using a pinched-expanded channel with a bifurcated inlet.¹⁹ In the following year, they fabricated a microfluidic chip with a main channel and multiple branch channels to separate cells with different diameters, proposing the concept of “hydrodynamic filtration.”²⁰ Both methods demonstrate stable behavior of particles with regular shape. However, because EPs have a wide range of diameters and a thin thickness that is comparable to sperm’s size, these two methods alone are not suitable for separating sperm from EPs.

Here, the pinched-expanded channel and the branch channels of the two methods above were combined and applied for the separation of sperm and EPs for the first time. A microfluidic device based on the hydrodynamic effect of flow at low Reynolds number was fabricated for the separation of sperm from mixed cells in forensic samples. The device utilizing the combined effect of the two methods provides a cost-effective and easily automated method for cell separation. Finite element simulation analysis was used to optimize the microchannel design to achieve a specific streamline distribution in the microdevice. After being introduced into the optimized microchannel, sperm cells and EPs flowed along different streamlines and, ultimately, entered different outlets. STR analysis was then performed to evaluate product purity, and the profiles demonstrated that a highly purified male fraction was obtained in the sperm outlet reservoir.

II. MATERIALS AND METHODS

A. Mock sexual assault sample preparation

Vaginal EPs in forensic samples were imitated with buccal EPs obtained from cotton swabs. Human semen samples were obtained from healthy donors in the Center of Reproduction Medicine, Peking University Third Hospital. EPs and sperm cells were diluted in PBS (phosphate-buffered saline, Sigma-Aldrich, St. Louis, MO) as a mock sample with a

concentration of 1000 and 300 cells/ μl , respectively. Cell concentration was determined with a hemacytometer (XB-K-25, Qiujiang, Shanghai, China).

B. Modeling and simulation

Computational fluid dynamics simulations were performed using COMSOL 4.3b (Comsol AB, Burlington, MA). Three-dimensional (3D) finite element geometry was built based on variable parameters and analyzed using the laminar flow module. The module is described by the Navier-Stokes equations with a no-slip boundary condition. The flow was considered to be incompressible. We optimized the channel design iteratively by altering the variable parameters of the geometry. Matlab 2011b (Mathworks, Natick, MA) was used for the iteration to calculate the optimal parameters. The Comsol with Matlab interface was used to implement the iterative analysis.

C. Microdevice fabrication

The microdevice consisted of an upper polydimethylsiloxane (PDMS) layer and a lower glass layer. The PDMS layer was fabricated following standard photolithography and rapid molding procedures.²¹ Briefly, SU-8 photoresist (MicroChem, Newton, MA) was patterned onto a silicon wafer to form a master with a chromium plate as a mask. PDMS prepolymer mixed 10:1 with a curing agent (Sylgard 184, Dow Corning, Midland, MI) was poured onto the master and cured at 72 °C for 90 min. The PDMS replica was then peeled off of the master, and the areas with functional microstructures were cut out. The inlet and outlet holes were punctured using a hole-puncher (18 gauge for inlets and 11 gauge for outlets, Wenhao Chip Technology, Suzhou, China). Both layers were treated with oxygen plasma (Femto, Diener Plasma-Surface-Technology, Germany) and irreversibly bonded together.

D. Experimental setup

Mock sample and PBS were introduced into the chip using syringe pumps (PHD 2000, Harvard Apparatus, Holliston, MA). Polytetrafluoroethylene (PTFE) tubing (0.5 mm ID) was utilized to connect the chip and the syringe (8.5 mm ID for the sample solution and 14.5 mm ID for PBS). The motion of cells was observed under an inverted microscope (AE31, Motic, Xiamen, China) with a 10 $\times$ objective lens (NA=0.25), and images of the separation process were monitored and recorded with a high-speed camera (MotionBLITZ Cube 3, Mikrotron, Germany) and desktop PC. To clearly observe the sperm tracks, an inverted microscope (1X71, Olympus, Tokyo, Japan) with a 20 $\times$ objective lens (NA=0.40) and a CCD camera (Clara, Andor, Belfast, UK) were used to record fluorescence micrographs of the sperm cells stained with propidium iodide (PI, Ebioscience, San Diego, CA).

E. Post-separation sample processing

The purity of the separation products was analyzed by their STR profiles, which were used to determine the source when compared to the reference sample. DNA extraction of the cells recovered in the outlet reservoir was performed using a MagAttract DNA Mini M48 Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. STR amplification was performed using an AmpFISTR[®] Identifier[®] Plus Kit (Applied Biosystems, Shanghai, China) in a reaction volume of 10 μl on a GeneAmp[®] PCR System 9700 (Applied Biosystems, Shanghai, China). The amplification program consisted of an initial denaturation at 95 °C for 11 min, followed by 29 cycles of amplification (94 °C for 1 min, 59 °C for 1 min, and 72 °C for 1 min), and a final extension at 60 °C for 60 min. The PCR amplification products were separated on a 3130xl Genetic Analyzer (Applied Biosystems, Shanghai, China), following the manufacturer's protocol to obtain the STR profile. The profile contained the following 16 loci: D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, Amelogenin, D5S818, and FGA.

III. RESULTS AND DISCUSSION

A. Microdevice design

EPs are squamous cells with a diameter of 40–60 μm , and sperm cells are tadpole-shaped with a head diameter of 4–6 μm . Because EPs have a thickness that is comparable to a sperm's size, the actual size difference between the two types of cells is small.

As far as “pinched flow fractionation”¹⁹ is concerned, as mentioned above, several pinched-expanded structure units have to be cascaded to achieve a precise separation of sperm and EPs, resulting in too many bifurcated inlets and thus complicated fluid control. Similarly, “hydrodynamic filtration”^{20,22} requires dozens or even hundreds of branch channels to first align the cells and then recover them, which makes the microchannel structure rather complex.

Here, the effect of the two methods was combined in a microdevice to achieve a size-based separation of sperm and EPs. The microdevice shown in Figure 1(a) cascaded in two parts, which are the pinched-expanded channel structure in the “pinched flow fractionation” (part I) and the branch channels in the “hydrodynamic filtration” (part II), respectively. First, the pinched-expanded structure, which can directly handle cells at a high concentration, was used to recover most of the sperm and deplete most of the EPs. Some sperm cells were considered a compromise in terms of the purity and failed to be recovered at this step. Meanwhile, these sperm cells and some EPs flowed into the main channel of part II and were aligned properly based on their different diameters under the effect of the pinched-expanded channel. Then, because sperm cells were aligned near the branch channels and EPs were aligned away from the branch channels, sperm could be further recovered in part II with a high level of purity.

More specifically, Figures 1(c) and 1(d) show a schematic of the separation process. In part I, there was a pinched and an expanded segment. When the mixture of EPs and sperm cells was introduced into the pinched segment from inlet 1, all cells were forced into alignment on one sidewall, regardless of their size, by the cell-free buffer from inlet 2. It was assumed that particles could not be placed geometrically within a certain distance from the sidewall, one that was equal to their radius. Because the aligned cells had different radii, their barycenters lied on different streamlines. Assuming that particles flowed along their underlying unperturbed streamlines at low Reynolds number, the slight differences in cell positions in the pinched segment were significantly amplified when the streamlines dispersed as the channel expanded. At a certain hydraulic resistance distribution, the underlying streamlines of sperm were within a threshold w from the upper sidewall in the pinched segment and then dispersed in the channel linked to outlet 1. Conversely, the underlying streamlines of EPs or other particles with larger diameters were beyond w from the sidewall and dispersed in other channels. As a result, sperm cells would flow along their underlying streamlines into the upper outlet 1, and EPs or other particles would be collected by other outlets as shown in Figure 1(d).

In part II, a series of channels branched off of the main channel, linking outlet 2 to further collect the sperm that was not recovered by part I. To collect enough sperm cells and lower the complexity of the channel structure in the same time, the number of branches was set to 8. The principle in part I was also applied during this portion of the separation. In this phase, sperm cells were aligned to one sidewall as a result of the effect of part I and the former branch channels they had passed by. At a certain hydraulic resistance distribution, the streamlines that the barycenters of the sperm cells lied on were within a threshold w from the sidewall and just went through the branch channels. In contrast, the streamlines over which the barycenters of larger particles lied were beyond w and went through the main channel. Consequently, sperm cells would flow into the branch channels, and other larger particles such as EPs would remain in the main channel as shown in Figure 1(e). Additionally, the right angle connection between the main channel and a branch channel was transformed into a rounded connection to smooth the flow direction change in the branch point and ensure that the sperm cells would enter the branch channel smoothly.

FIG. 1. Microdevice used to separate sperm from EPs. (a) Schematic illustration of the microchannel design, (b) top view of the microdevice, (c) detailed illustration of part II shown in (a), (d) detailed illustration of part I shown in (a), and (e) detailed illustration of the branch points shown in (c). I1, I2, O1, O2, O3, and O4 indicate inlet 1, inlet 2, outlet 1, outlet 2, outlet 3, and outlet 4, respectively.

B. Optimization of microchannels and finite element analysis

The hydraulic resistance distribution design based on the resistive circuit analysis has low accuracy.^{20,23} Thus, researchers have used computational fluid dynamics analysis in microstructure design more frequently to obtain a more accurate and rapid solution.^{24,25} In this work, Comsol was used to obtain the optimal hydraulic resistance distribution in the microchannel. We iteratively optimized the channel design by altering variable parameters of the geometry. As the hydraulic resistance of part I and part II differed significantly, the hydrodynamic influence between them could be disregarded. Consequently, these two parts were analyzed

independently. The boundary conditions for the inlets assumed a set velocity. The normal inflow velocity of the inlet in part I and part II was set to 0.01 m/s and 0.25 m/s, respectively. The boundary conditions for the outlets assumed a set pressure and vanishing viscous stress. The pressure at the outlets was set to zero in both parts. We applied the no-slip condition to all other boundaries.

Here, we take the design of part II as an example to illustrate the designing process. The flow regime in the microfluidic channels was laminar flow. According to the study of Brody *et al.* (1996), the analytical solution of the flow depth average velocity based on the Navier-Stokes equations in the rectangle channel can be expressed as

$$\bar{u}(x) = G \sum_{l=0}^{\infty} \frac{1}{(2l+1)^4} \cdot \left[1 - \left(\frac{\cosh((2l+1)\pi x/h)}{\cosh\left(\frac{(2l+1)\pi d_0}{h}\right)} \right) \right], \quad (1)$$

where G is a constant relevant to the physical properties of the fluid, x is the position coordinate along the width of the channel as shown in Figure 1(e), h is the depth of the channel, and d_0 is the width.²⁶ Then, the volumetric flow rate ratio of the fluid within w from the upper wall in the main channel to the total volumetric flow rate Q_0 is

$$\frac{Q_w}{Q_0} = \left(\int_w^{\frac{d_0}{2}} \bar{u}(x) \right) / \left(\int_{-\frac{d_0}{2}}^{\frac{d_0}{2}} \bar{u}(x) \right). \quad (2)$$

On the other hand, the volumetric flow rate ratios distributed into the corresponding branch channels calculated by Comsol are Q_i/Q_0 ($i = 1, 2, \dots, 8$). When the computational analysis results Q_i equaled the analytical result Q_w , which can be expressed as

$$\frac{Q_i}{Q_0} = \frac{Q_w}{Q_0} (i = 1, 2, \dots, 8), \quad (3)$$

it was considered that the fluid within w from the wall flows into the branch channels. In other words, the streamlines within w go through the branch channel, and the other streamlines beyond w remain in the main channel. Assuming that particles flow along their underlying streamlines, sperm cells with radii smaller than w tended to flow into the branch channel, and EPs with radii larger than w tended to remain in the main channel as described above. Here, w was set to $3.5 \mu\text{m}$ to guide as many sperm cells as possible into the sperm outlet while still ensuring purity.

The design objective was meant to ensure that an equal volumetric flow rate Q_w was distributed into each of the branch channels, i.e., to make Eq. (3) true. Parameters, except for the lengths of the narrow segments h_i ($i = 1, 2, \dots, 8$), were set as definite numbers. h_i , as shown in Figure 2(a), was set as a variable parameter. The 3D finite element model shown in Figure 2(a) was built upon these parameters. The depth of the microchannel was set to $50 \mu\text{m}$ and fabricated at $52.6 \mu\text{m}$, making the short axis of EPs parallel to the depth direction. The width d_0 was set to $150 \mu\text{m}$. Thus, according to Eq. (2), $Q_w/Q_0 = 0.0024$. Each branch channel consisted of a narrow segment and a broad segment, 16 and $32 \mu\text{m}$ wide, respectively. The hydraulic resistance of the branch channel increased as the length of the corresponding narrow segment increased. Thus, the hydraulic resistance distribution within all branch channels and the main channel, as well as Q_i , were adjusted by changing h_i . The interval between the narrow segments was $80 \mu\text{m}$. The overall length of the branch channels was $3500 \mu\text{m}$.

The optimal h_i , which made Eq. (3) true, was calculated by means of the iterative method. The initial lengths of the narrow segments of the branch channels were set to 3400, 3000, 2500, 2000, 1500, 1000, 500, and $100 \mu\text{m}$, respectively, and the iterative formula was

FIG. 2. (a) and (b) The 3D geometry of part II (a) and part I (b) built in Comsol. (c) and (d) Corresponding finite element simulation results of slice velocity of part II (c) and part I (d). The red lines indicate streamlines.

$$\begin{cases} h_{i_{n+1}} = h_{i_n}(1 + s \cdot er_{i_n}), i = 1, 2, \dots, 8 \\ er_{i_n} = \frac{e_{i_n} - e_0}{e_{i_n}} \\ e_{i_n} = \frac{Q_{i_n}}{Q_0}, \end{cases} \quad (4)$$

where the iterative constant $s=0.5$. This iterative process continued until the termination condition was met,

$$\text{err}_n = \sum_{i=1}^8 (e_{i_n} - e_0) < 5 \times 10^{-9}, \quad (5)$$

where err is the iterative error.

The iterative process stopped after 13 iterations. The simulation results of part II are shown in Figure 2(c), and the variations of h_i and err in the iteration process are shown in Figure 3. The optimal narrow segment lengths of each branch channel h_i were 2947, 2674, 2408, 2153, 1910, 1678, 1453, and 1220 μm , respectively.

In part I, to allow cells to flow without clogging, the pinched segment was set to 80 μm in width and 150 μm in length. In the same way, the hydraulic resistance distribution between the four channels linking the four outlets was adjusted by manually changing their width and length, making $Q_i = Q_{wi}$ ($i = 1, 2, 3$, and 4). The channel linking outlet 1 in part I was equivalent to the branch channel in part II.

Figure 2(b) shows the 3D finite element model of part I. The threshold w of outlet 1 and outlet 2 was set to 4 μm and 14 μm , respectively. The corresponding volumetric flow rate ratios distributed into outlet 1 and outlet 2 to the total flow rate were 0.008 and 0.086, respectively. For outlet 1, the given w was set slightly larger than the radius of the sperm head to recover as many sperm cells as possible. For outlet 2, w was the balance between guiding all of the sperm

FIG. 3. Variation of the narrow segment length of the branch channels h_i (a) and volumetric flow rate iterative error err (b) in the iteration process.

having failed to be recovered by part I into part II and letting as few EPs as possible enter. The average width of the four outlet channels was 50, 150, 400, and 400 μm , from the upper channel to the lower channel, respectively. The average length was approximately 6900, 2500, 2450, and 2450 μm , respectively. The simulation results are shown in Figure 2(d).

It should be noted that particles do not strictly flow along their underlying streamlines. Several forces may affect a cell's behavior in the flow field. First, the shear gradient lift force and the wall effect lift force,¹⁷ which are widely considered in inertial microfluidics, may cause cross-streamline migration of particles and lead particles away from the wall. The influence of these forces becomes stronger as the Reynolds number increases. The maximum Re existed in the pinched segment of the microdevice. Cells that were aligned to the sidewall in this region had a tendency to flow across the streamline away from the wall. As a result, we set the length of the pinched segment to be relatively short and the buffer's flow rate relatively low. Second, when a particle is in the vicinity of a bifurcation, the pressure difference on each side of the particle would attract it to enter the branch channels.²⁷ Thus, a particle with a radius slightly larger than w may enter the branch channels as well. Consequently, although we set w to be larger than the radius of a sperm cell to recover as many sperm cells as possible, w could not be too big to prevent small EPs from entering the sperm outlet. Here, the most critical factor is the purity of the product in the sperm outlet, which may tremendously impact the gene analysis result in forensic cases. Thereby, the flow paths of some sperm cells were considered as a compromise.

C. Separation behavior of sperm and EPs

The sperm and EPs in the mixed solution were introduced into the microdevice from inlet 1 at a fixed throughput of 2 $\mu\text{l}/\text{min}$. The PBS was introduced from inlet 2 at a flow rate increasing from 15–75 $\mu\text{l}/\text{min}$ with an interval of 15 $\mu\text{l}/\text{min}$. During the experiments, we found that all of the particles were tightly aligned to one sidewall until the buffer's flow rate was $> 60 \mu\text{l}/\text{min}$. Therefore, the buffer's flow rate was set to 60 $\mu\text{l}/\text{min}$ for the followed experiments. The ratio of the flow rates at the two inlets assured that all cells were aligned to the sidewall.

Figure 4 shows the behavior of EPs in the microdevice. Most of the EPs flowed into outlets 3 and 4, while the rest of them entered outlet 2. Simultaneously, a great number of sperm cells flowed into outlet 1, and the sperm cells that were not recovered by outlet 1 entered into part II. Most of these sperm cells were eventually successfully recovered by outlet 1 through the eight branch channels. The tracks of the sperm cells in parts I and II are shown in Figure 5. As shown in Figure 5(b), some sperm cells were stuck in the right side fillets of the branch points due to the low flow rate in this region. This phenomenon could be avoided by increasing the size of the fillets. The experimental results demonstrated that the tracks of the cells matched well with the analytical streamlines shown in Figures 2(c) and 2(d).

D. Separation performance analysis

The 50- μl mock sample was processed in one experiment at a throughput of 2 $\mu\text{l}/\text{min}$. To further test the purity of the separated sperm fraction, a representative product collected from outlet reservoir 1 was processed via post-separation sample processing. Alleles of the semen donor and EPs donor, respectively, had their specific repeat number at each loci in the STR profiles. The STR analysis results, as shown in Figure 6, indicated that an independent STR profile for the sperm could be obtained. Additionally, assuming the amplification efficiency of each allele was the same, the percentage of male DNA fraction can be calculated by the peak value of the specific alleles of the sample donors. Thus, by comparing the STR profiles of the semen donor (Figure 6(b)) and EPs donor (see Figure S1 in the supplementary material, Ref. 31) and calculating the average peak value ratios of the male and female contributors for the STR loci, the percentage of the male DNA fraction in the mixed sample and the separated collection were determined to be $20.3 \pm 12.1\%$ and $94.0 \pm 4.7\%$, respectively. Furthermore, we processed the 50- μl same mock sample using another microfluidic chip as a control. The structure of the control chip contained only part I, as shown in Figure S2 (supplementary material,

FIG. 4. Micrographs showing the behavior of EPs. (a) EPs were aligned to the sidewall in the pinched segment shown in Figure 1(d). (b) EPs flowed into the channel linking outlet 2 or outlet 3 in the expanded segment shown in Figure 1(d). (c) EPs flowed through the main channel in part II shown in Figure 1(c). The scale bars indicate 100 μm . Dashed lines indicate the tracks of EPs. The outlines of EPs are highlighted with yellow lines.

Ref. 31). The percentage of the male DNA fraction in the separated collection was $97.0 \pm 2.3\%$.

To evaluate the sperm recovery efficiency, a FACS (BD FACSAria III, BD Biosciences, San Jose, CA) and TrucountTM tubes (BD Biosciences) were used to count the number of sperm cells in different collections and the mixed samples prior to separation. The sperm recovery

FIG. 5. Fluorescence micrographs of sperm flowing through the microchannel in part I (a) and part II (b). White bright lines indicate the tracks of sperm cells. Channel walls are visualized with dashed lines.

FIG. 6. The STR profiles indicated the purity of the separated collection. (a) STR profile of the sample prior to separation, (b) a STR profile of the semen donor, and (c) a STR profile of the product collected from sperm outlet reservoir (outlet 1) of the microdevice.

efficiency, which is the ratio of the two numbers above, was $41.1 \pm 2.9\%$ ($n=3$). A small number of sperm cells were stuck in the channel or adhered to EPs. This problem can be overcome by channel surface modification and buffer ingredient variation. Furthermore, the sperm recovery efficiency of the control chip was $26.9 \pm 0.7\%$ ($n=3$). The results showed that part II

increased the sperm recovery efficiency dramatically, while it did not decrease the purity of the separation product.

The total separation time for the 50- μ l of sample was $\sim$ 0.5 h, which is much shorter than 3 h when using the conventional DE method and could be further reduced by integrating more parallel separation units. Compared to other alternative methods,^{4,7,8} the proposed microdevice has the following four advantages: (1) because this microfluidic chip does not contain any extra components such as microelectrodes or piezo transducers, the fabrication process is simple and the cost is low; (2) the device eliminates the need for complicated external instruments, aside from two pumps for fluid control, making the device easy to automate and miniaturize; (3) the chip material (PDMS) can be replaced with polymethyl methacrylate or other polymer materials that are easy for mass production,²⁸ and thus the microfluidic chip has a great potential to be transformed into a disposable product for forensic analysis of sexual assault cases; and (4) the microdevice is easily integrated, and the integration of this microfluidic chip with subsequent sample processing chips (e.g., DNA extraction, PCR, or STR chips^{29,30}) may offer a chance to replace the conventional labor-consuming and time-consuming processes for forensic evidence analysis.

IV. CONCLUSION

In this work, we developed an innovative microdevice for separating sperm from EPs using the hydrodynamic effect at low Reynolds number. A pinched-expanded channel and a series of branch channels were combined in the device, where sperm cells and EPs were aligned to one sidewall and afterward flowed into different outlets. Sperm were successfully separated from a 50- μ l mock sample within 0.5 h. The STR profiles of the products demonstrated that a highly purified male fraction could be obtained (94.0% male fraction). The microdevice is easily operated and has a simple structure and fabrication process, making it a cost-effective and easily automated tool for the separation of sperm from EPs. Additionally, the microfluidic chip can be integrated with other chip-based processing steps, such as DNA extraction, PCR, or STR, to form a fully integrated lab on a chip system for forensic analysis of DNA.

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