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Adhesion based detection, sorting and enrichment of cells in microfluidic Lab-on-Chip devices

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The detection, isolation and sorting of cells are important tools in both clinical diagnostics and fundamental research. Advances in microfluidic cell sorting devices have enabled scientists to attain improved separation with comparative ease and considerable time savings. Despite the great potential of Lab-on-Chip cell sorting devices for targeting cells with desired specificity and selectivity, this field of research remains unexploited. The challenge resides in the detection techniques which has to be specific, fast, cost-effective, and implementable within the fabrication limitations of microchips. Adhesion-based microfluidic devices seem to be a reliable solution compared to the sophisticated detection techniques used in other microfluidic cell sorting systems. It provides the specificity in detection, label-free separation without requirement for a preprocessing step, and the possibility of targeting rare cell types. This review elaborates on recent advances in adhesion-based microfluidic devices for sorting, detection and enrichment of different cell lines, with a particular focus on selective adhesion of desired cells on surfaces modified with ligands specific to target cells. The effect of shear stress on cell adhesion in flow conditions is also discussed. Recently published applications of specific adhesive ligands and surface functionalization methods have been presented to further elucidate the advances in cell adhesive microfluidic devices.

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1 Introduction

The isolation and sorting of cells is frequently used in laboratories for clinical diagnostics or basic research. Separation of human lymphocytes from blood for HIV diagnosis tests,[1–3] enrichment of stem cells,[4] and cytosensors for detection of different diseases[5,6] are some examples of the need for cell sorting. The fields of biology and biotechnology are the main users of sorting techniques and devices for isolation of rare cell populations. The importance of cell sorting has not been solely limited to biology and medicine but also analysis and subsequent cultivation of desired cells from a defined cell population in the fields of molecular genetics, diagnostics and therapeutics. On the other hand, use of pure cells help reduce variations among experiments and thus expedite scientific discovery.

Cell sorting technique is defined as any procedure which can be used for separation, isolation or enrichment of a specific cell

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type.[7] In this technique, either physical properties, such as density or size, or affinity such as electric, magnetic or adhesive properties specific to each cell type is used to sort cells. Conventional techniques for the separation of cells include filtration, centrifugation and sedimentation, which are carried out either in a batch or continuous manner and can be easily implemented in large-scale operation. However, where cell size differences are not significant, effective separation is impeded. This issue becomes more significant when small sample volumes are employed. The other conventional techniques for cell sorting are: panning,[1] fluorescence activated cell sorting (FACS),[8] and magnetic activated cell sorting (MACS).[9]

Recent developments in lab-on-a-chip devices have opened new avenues for the analytical study of biological and chemical samples in a single microfluidic device, and have begun to play an increasingly important role in cell biology, neurobiology, pharmacology, and tissue engineering. The cell sorting technology has also started to benefit from the major advantages of microfluidic devices, namely the ability to design cellular microenvironments,[10] precise control of fluid flow,[11] and reduction of time and cost of cell culture assays.[10] Many microfluidic devices with different flow channel designs have been introduced as cell sorting devices based on the physical properties. Physical-based cell sorting methods such as filtration,[12,13] sedimentation and hydrodynamic force[14–16] are mainly limited to the differences in size or density of the target cell(s) and other cell types within a mixture. A comprehensive review of these devices has been published by Kim *et al.*[11] Compared to physical-based sorting methods, affinity-based techniques include a wide array of approaches, taking advantage of electric, magnetic, optical and adhesive properties of cells. FACS,[17–21] MACS,[22–24] acoustic cell sorting,[25–28] optical cell sorting,[29,30] dielectrophoresis (DEP)[31,32] and adhesion-based sorting are among those devices which provide more flexibility and precision in targeting and separating desired cell types. Table 1 depicts cell sorting techniques implemented in microfluidic chips.

Based on the detection method used, cell sorting microfluidic devices can be divided into label-free and pre-processed techniques. In latter techniques which include FACS and MACS, desired cells are tagged with fluorescent dyes or magnetic beads respectively and are exposed to electrical or magnetic fields. Although in label-free techniques, such as filtration and hydrodynamic separation, there is no need for a pre-processing step, the sorting options are limited to physical properties or external forces, such as acoustic or optical fields. In contrast to label free methods, pre-processed techniques might alter the separated cells' functions due to the presence of attached magnetic beads (MACS) or antibodies (FACS). This might be a great concern if the isolated cells are designated to specific applications such as tissue regeneration. Adhesive-based sorting devices are among the few label-free sorting techniques which are not dependent on the size of the particles, making them a suitable option for cell sorting. The significance of adhesive based separation is not only attributed to its label free nature, but also to its ability to achieve specific adhesion of target cell types on the surface of microchannels. This paper reports on adhesion based microfluidic devices for sorting different cell types, enrichment of rare cells and the detection and diagnostics of diseased cells. Despite the availability of several review papers dedicated to cell sorting principles[11,21,32,37–39] there is so far no review with a primary focus on adhesion-based cell sorting microchips. Herein, surface patterning and modification techniques for microfluidic devices as well as the effect of shear stress on cell adhesion are discussed. Particular emphasis is on the new trends for separation or detection of desired cell types using cell-specific adhesion molecules including proteins, peptides and aptamers.

2 Factors affecting cell adhesion in microfluidic devices

Cell adhesiveness is cell type dependent. This feature has been used to develop multiple techniques and devices to study the adhesion properties of different cell types.[40–44] With the recent progress in the development of microfluidic devices, their use is frequently investigated for the separation of various cell types in one device using parallel channels modified with different molecules. This reduces tremendously the assay time due to higher surface contact between target cells and modified surfaces.

Shear stress and flow rate, surface topography, concentration and specificity of cell adhesive molecules, are main factors affecting cell adhesion in microfluidic devices. However, the surface properties represent the main driving force that influences the composition and orientation of adsorbed proteins and subsequent cellular events, such as adhesion, proliferation, and differentiation.[45] Common surface modification approaches consist of micro/nano structuring of the surface and/or functionalization with specific ligands. These ligands referred to as cell adhesion molecules are involved in cell-cell and cell-surface interactions and are commonly used in the design of sorting, sensing and diagnostic devices to target desired cell types. Not all cell adhesion molecules have the same strength of binding, some are strong and some are weak and dynamic.[46] Nevertheless, there are many unknowns about cell adhesive molecules, particularly about the role of mechanical and chemical signals generated by them to facilitate the crosstalk between cell and extracellular environment.

2.1 Preparation of cell-adhesive surfaces

Optimal surface properties, in terms of topography and roughness, as well as the homogeneous distribution of ligands on the surface, can significantly enhance the efficiency and sensitivity of the device. Consequently, different techniques have been implemented for surface modification including micro/nano patterning and adhesive ligand coating.

Conventional microfabrication techniques such as photolithography, soft lithography,[47] electrochemical discharge machining[48] and deep reactive ion etching[49] are used for patterning micro structures onto surfaces. Several other methods including electron beam lithography,[50] electrochemical and chemical etching,[51] UV-assisted capillary molding,[52] and layer by layer technique[53] are used for patterning nanostructures on microchannel surfaces. Silanization,[54,55,56] microcontact printing[57,58] and microfluidic printing[59,60] are three main techniques for immobilization of adhesive ligands onto surfaces of microchannels.[2,6,61,63] Table 2 compares the advantages over the disadvantages of these three methods for protein/peptide/aptamer modification on microchannel surfaces. Combination of

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Table 1 Microfluidic devices developed for cell sorting

Sorting method	Separation property	Limitations	References
FACS[a]	Fluorescence	False detection as separation rate increases, pre-processing is necessary, expensive	17,18,33,34
Hydrodynamic separation	Size	Selectivity is only based on the size of the particles, efficiency is an issue	14–16,35
Physical trapping (Filtering)	Size	Selectivity is only based on the size of the particles, needs complex microfabrication	12,13,36
DEP[b]	Dielectric particles	Requires external electric field	31,32
Acoustic cell sorting	Size	Particle diameter must be less than half of the applied wavelength, requires external field	25–28
Optical forces	Optical polarizability	Based either on size or index of refraction	29,30
MACS[c]	Magnetic properties	Needs external magnetic field and pre-processing	22–24

[a] FACS: Fluorescence activated cell sorting. [b] Dielectrophoresis. [c] MACS: Magnetic activated cell sorting.

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different methods has also been used to achieve better adhesive properties.[64,65] Micro/nano structuring of the surface followed by functionalization with the biomolecules of choice[50] or functionalizing micro/nano particles with the adhesive biomolecules[66] were showed to improve adhesive properties.

3 The effect of flow and shear stress on cell adhesion

The main difference of cell adhesion in microfluidic devices compared to conventional cell adhesion flasks is the flow characteristics and the resulted shear stress exerted on the suspended cells. Different groups have reported adhesion and detachment of different cell types under varying shear stresses.[67–74] The first device for systematically investigating the effect of shear stress on cell adhesion was introduced by Shunichi *et al.*[75] Based on the Hele-Shaw flow theory between parallel plates, the authors fabricated a nozzle shaped microfluidic channel in which shear stress decreased linearly along the channel length (Fig. 1).

According to eqn (1), shear stress exerted on the suspended particles depends on the geometry of the channel (w: inlet width, L: length and h: depth), volumetric flow rate (Q) and viscosity of suspension (μ):

$$\tau_{\omega} = \frac{6\mu Q}{wh^2} \quad (1)$$

Using this design, the group showed that the adhesion of platelets on fibrinogen coated glass surfaces was shear dependant.[75] This design has been used by other groups[3,63,76] to study cell adhesion under different shear stresses to obtain optimum adhesion conditions.

In general, increasing the shear stress decreases the adhesion rate. This is an important consideration in designing a microchannel for separating different cell types at different local points of a microchannel. The results for adhesion of smooth muscle cells and endothelial cells on specific peptides indicate that the number of cells adhering on the surface strongly depends on shear stress.[63] For instance, in a suspension of mixed endothelial cells (ECs) and smooth muscle cells (SMCs), 83% of the SMCs adhered onto the surface coated with their specific peptide (VAPG) at a shear stress of 2.9 dyn/cm^2 while the highest adhesion rate (86%) for ECs was achieved at a shear stress of 1.9 dyn/cm^2.[63] For endothelial progenitor cells (EPCs) the optimum shear stress was reported to be 1.47 dyn/cm^2.[62]

Microchannel geometry also plays an important role in cell adhesion and should be taken into consideration, particularly for subsequent modification of the device to introduce adhesive regions on the channels surface. Eqn (1) depicts the shear stress along the axis passing through the center of the microchannel (Fig. 1). Shear stress also varies along the microchannel's width especially when turns are included in the design. In general, channels with sharp turns are not optimal for cell adhesion. When turns are needed in the design, they should be curved to obtain uniform velocity. For straight microchannels, cell adhesion is independent of the length of the microchannel up to 100 mm. For capturing rare cell types where a small adhesion surface is needed, straight microchannels are better options. When a large amount of cells and a large surface area is required for adhesion, spiral shape microchannels are alternative choices as the shear stress remains almost constant in the entire width.[70]

As of yet, there remains a need for a comprehensive report on the detachment of adhered cells by shear stress and flow rate. Zhang *et al.*[43] have investigated Chinese hamster ovary (CHO) cell detachment from surfaces modified with collagen, silane and glutaraldehyde under different shear stresses. Cells, adhered on non-treated glass surface, detached easily from the surface, while collagen coated surface had the highest adhesion force. The authors have also shown the effect of shear stress on the detachment of Chinese hamster ovary (CHO), T47D, NCTC 2544, CaC02 and U937 cell lines from silane coated glass surface. CHO cell line needed the lowest shear stress for detachment whereas U937 cell line required the highest detachment shear stress. Interestingly, all cell lines and surfaces had almost the same detachment percentage when the shear stress increased to more than 100 dyn/cm^2.

Lu *et al.*[68] also investigated fibroblast adhesion onto surfaces coated with different concentrations of fibronectin under different

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Table 2 Comparison of surface modification techniques for peptide/protein/aptamer immobilization

Surface modification technique	Advantages	Disadvantages
Silanization	Reliable, Easy procedure,	Long process, Needs an oxygen free atmosphere, Needs subsequent assembly for use in microfluidics, Not selective,
Micro-contact Printing	Fast, Applicable to a variety of patterns, Repeatable, Selective,	Needs more precision, Needs to be assembled later for use in microfluidic, Difficult to control ligand concentration
Microfluidics	Is performed on the final microfluidic device, Fast, No sterilization needed, Selective, Controllable ligand density	Needs oxygen free atmosphere, Patterned geometries are limited to open network structures

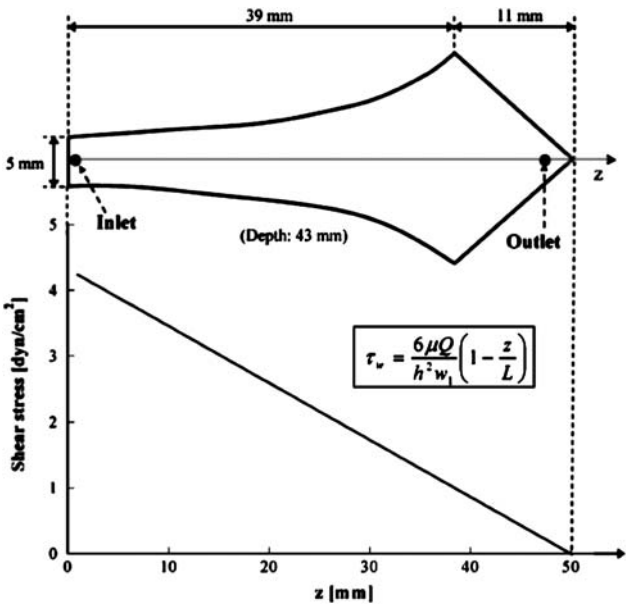

Fig. 1 Flow chamber geometry and shear stress profile.[3] The relationship between shear stress (τ_w) and axial position (z) is given by the equation derived by Shunichi *et al.*[75] (Reproduced from ref. 3 with permission from American Chemical Society.)

shear stresses using microfluidic platforms and showed that detachment rate decreases by increasing the concentration of cell adhesive ligand on the surface. Fibroblast detachment was about 10% at high concentrations of adhesive ligand (10 μg/ml) while almost all of the adhered cells were detached at lower ligand concentration (0.1 μg/ml) under a relatively low shear stress.

In a flow acceleration assay, low flow acceleration (0.032 ml/min^2) induced the deformation of prostate cancer cells attached on a protein-modified surface, while at higher flow acceleration rate (0.32 ml/min^2) the deformation was negligible and the detachment was significant.[69]

4 Adhesion based microfluidic cell sorting devices

4.1 Cell adhesion on micro/nano structured surfaces

Cell adhesion, separation and proliferation can significantly increase by changing the surface topography.[50,53,77,78] Adhesion of COS-7 fibroblasts on PDMS surfaces modified by Al microstructures[79] and mouse fibroblast NIH/3T3 cells on 300 nm wide and 150 nm high gold features[80] are examples of cell adhesion in micro/nano patterned microfluidic devices. Hengyu *et al.*[77] reported that the adhesion of Escherichia coli on micro/nano-textures patterned on glass surface is twice as high as on a smooth surface.

Separation of human breast cancer cells from epithelial cells in a microfluidic device with patterned microchannels was achieved by Kown *et al.*[78] Various nanostructures (pillar, perpendicular and parallel lines) of 400 nm were fabricated on PDMS surfaces using UV-assisted capillary moulding (Fig. 2). The adhesion strength of MCF10A cells was found to be higher than that of MCF7 cells regardless of pre-culture time and surface nano-topography at all flow rates. For the separation of cancer cells,

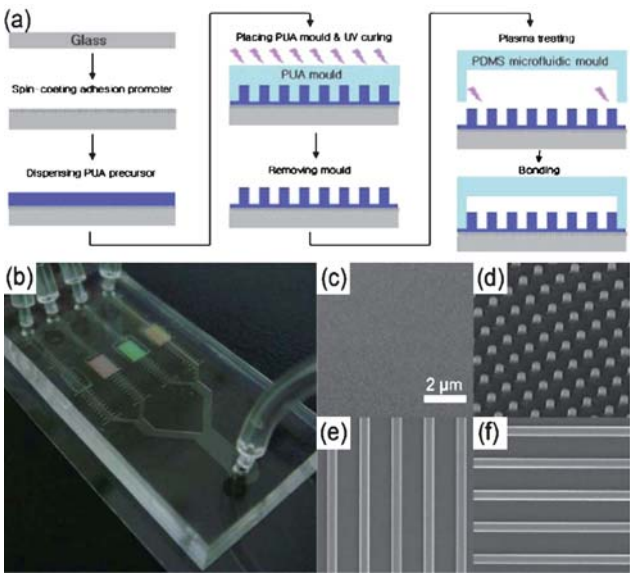

Fig. 2 (a) A scheme of fabrication of microfluidic channels integrated with a nanopatterned substrate. PUA nanostructures were fabricated using UV-assisted capillary moulding onto glass substrate. (b) Fabricated microfluidic channel. (c–f) SEM images of flat or three PUA nanostructures fabricated inside a microfluidic channel: (c) PUA flat surface, (d) 400 nm pillars (e) 400 nm perpendicular lines, and (f) 400 nm parallel lines. (Reproduced from ref. 78 with permission from Royal Society of Chemistry.)

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the optimum conditions were achieved by a 2-hour pre-culture on perpendicular line patterns (Fig. 2(e)) followed by flow-induced detachment at 200 μl/min. Applying these conditions, the fraction of MCF7 cancer cells in the effluent increased from 0.36 to 0.83. Although this device can enhance the enrichment of MCF7 cells from MCF10A cells, the long pre-incubation time (2 h), difficulty in controlling the experimental parameters to reach the optimum conditions, and low specificity of the device are the major limitations for its practical applications.

A new approach in adhesion based microfluidic devices which uses hybrid glass/gold micro/nanostructures was reported by Westcot *et al.*[51] Different patterns were tested for cell adhesion on this hybrid substrate (Fig. 3). Fibroblasts did not adhere to these surfaces with an $EG_4C_{11}SH$ (tetra(ethylene glycol) undecane thiol) monolayer on the surface, and interestingly, did not bind to the gold gradient region until HDT (hexadecanethiol) was added to the gold in the microchannel.[51]

4.2 Ligand-specific cell adhesion

Specific adhesion of cells on modified surfaces is a reliable and highly sensitive approach which has recently gained growing attention for separation of different cell lines. Specificity of cell adhesion in microfluidic devices can be achieved using proteins, peptides and aptamers to capture desired cell types. The key point, however, remains in finding of the biomolecule which is specific to the targeted cell membrane.

4.2.1 Protein-specific cell adhesion. One of the pioneering works pertaining to the capture and detection of circulating tumour cells (CTC) in a microfluidic device was reported by Nagrath *et al.*[6] Specific adhesive antibodies namely EpCAM and TACSTD1 which are both anti-epithelial cell-adhesion-molecules, were used to capture the CTCs (Fig. 4).

In two separate studies, Epithelial Membrane Antigen (EMA) and Epithelial Growth Factor Receptor (EGFR) were reported

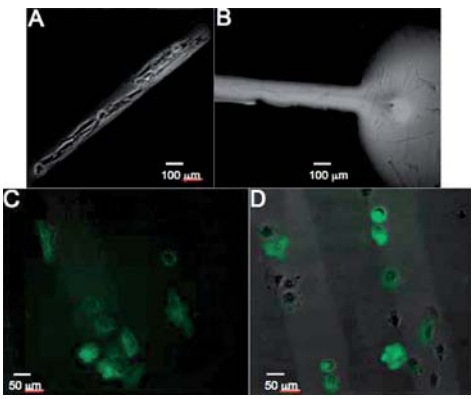

Fig. 3 Cell attachment to microfluidic generated partially etched and functionalized gold substrates. (A) Cells adhered specifically to the etched line pattern presenting HDT. (B) Cells adhered to a circular pattern. (C) Stably transfected Rat2 fibroblasts expressing GFPcoronin attached to the partially etched pattern. (D) The same fluorescent cells bound to a line pattern. The cells only adhere to partially etched regions of the gold allowing for live-cell visualization of cells on patterned surfaces. (Reproduced from ref. 51 with permission from American Chemical Society.)

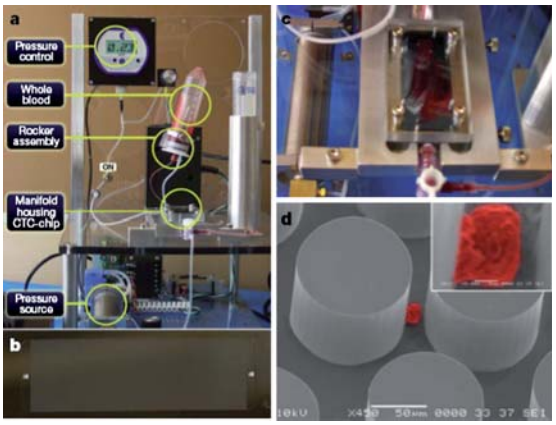

Fig. 4 Isolation of CTCs from whole blood using a microfluidic device. a) The workstation setup for CTC separation. The sample is continually mixed on a rocker, and pumped through the chip using a pneumatic pressure-regulated pump. b) The CTC-chip with microposts etched in silicon. c) Whole blood flowing through the microfluidic device. d) Scanning electron microscope image of a captured NCI-H1650 lung cancer cell spiked into blood (pseudo coloured red). The inset shows a high magnification view of the cell. (Reproduced from ref. 6 with permission from Nature publishing group.)

as potential capture antigens for breast cancer cells.[81,82] The specificity of EMA was between 60% to 78%[82] and EGFR was over expressed in 45% of breast cancer cells.[81] Du *et al.*[83] used this concept to fabricate a simple microfluidic device coated with EMA and EGFR to specifically capture breast cancer cells in a mixture with normal cells. The capture rate for breast cancer cells in the proposed microchip was more than 30% while less than 5% of the normal cells adhered to the surface after three minutes at a flow rate of 15 μl/min.

Separation of Endothelial progenitor cells (EPCs) and vascular endothelial cells (VECs) were also reported through the use of antibody modified surfaces. EPCs showed more tendencies to adhere to anti-CD34 modified surface and VECs adhered mainly to surfaces modified by anti-CD31.[62] Some non-specific adhesion was also reported in this work.

Combinations of different cell separation techniques can help increase the efficiency and specificity of separation. As an example, Hashimoto *et al.*[84] combined the principle of DEP with adhesion-based separation in a microfluidic chip to increase the reliability and specificity of the separation. The device was functionalized with anti-CD16b to selectively separate neutrophils from a suspension of mixed leukocytes (Fig. 5). After antibody immobilization, an AC voltage was applied to the microelectrode array to concentrate the neutrophils from the flowing suspension and subsequently capture the desired cell type by its specific antibody on the surface. This approach was also effective for capturing Eosinophil cells once anti-eosinophil was immobilized on the surface.

Another approach to the adhesion-based cell separation could be achieved by modifying micro and nano particles with the specific protein to trap the cells inside the flow. An interesting design for separation of A549 cancer cells, using a combination of modified magnetic nanoparticles and magnetic micropillars was reported by Liu *et al.*[66] The authors used photolithography to integrate Nickel micropillars on the microchannels surfaces.

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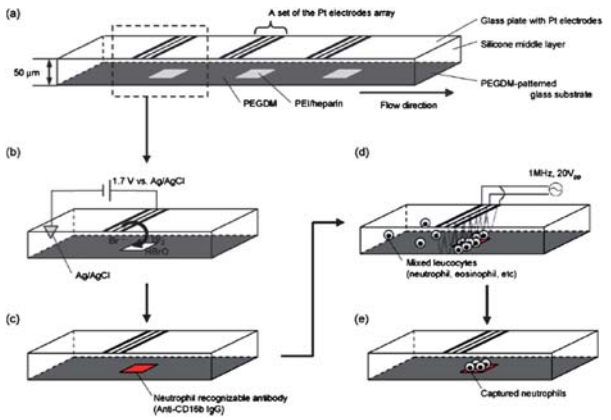

Fig. 5 Schematic representations of selective capture of neutrophils from mixed leukocytes within a microchannel. (a) Construction of the microfluidic device. A PEGDM-free region covered with PEI/heparin layer is positioned under a set of the Pt electrodes array. (b) The PEI/heparin layer is removed by HBrO generated at the central electrode to allow protein adsorption. (c) Neutrophil-specific antibody is immobilized on the protein-adsorptive region. (d) Leukocytes are concentrated at the antibody-immobilized region by negative DEP force. (e) Neutrophils are biochemically captured by the antibody and unbound cells are washed away. (Reproduced from ref. 84 with permission from ELSEVIER.)

A549 cancer cells attached to the supermagnetic beads, functionalized with WGA (Fig. 6) were trapped using a magnetic field created by the micropillars. A capture efficiency of 62% to 74% was achieved.

4.2.2 Peptide-specific cell adhesion. Patterning selected peptides can direct adhesion of specific cell lines exclusively to predetermined regions on microchannel surfaces. Recently Hasenbein *et al.*[85] used microcontact printing to pattern a surface with Arginine-Glycine-Aspartic Acid-Serine (RGDS), Lysine-Arginine-Serine-Arginine (KRSR), Arginine-Aspartic Acid-Glycine-Serine (RDGS) and Lysine-Serine-Serine-Arginine (KSSR) peptides. Following four hours incubation, adhesion of either osteoblasts or fibroblasts on surfaces patterned with the RDGS and KSSR was not significant but both osteoblasts and fibroblasts adhered and formed clusters onto areas modified with the adhesive RGDS, whereas only osteoblasts adhered and formed clusters onto the areas modified with KRSR.

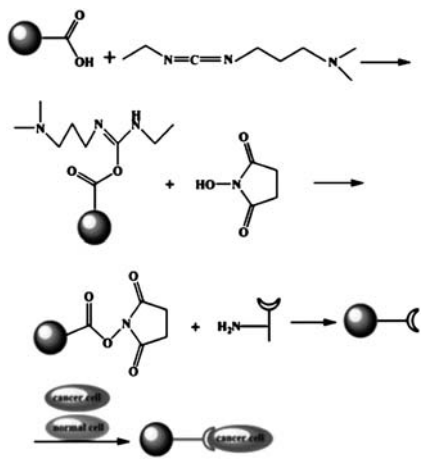

Fig. 6 Covalent binding of the specific protein to the magnetic beads and subsequent adhesion of the cancer cell. (Reproduced from ref. 66 with permission from Wiley InterScience.)

Plouffe *et al.*[63] used Arg-Glu-Asp-Val (REDV) and Val-Ala-Pro-Gly (VAPG) peptide sequences to separate smooth muscle and endothelial cells respectively in a microfluidic device. By controlling the shear stress and flow conditions a separation purity of 82% was obtained. KYSFNYDGSE peptide, a sequence of 10 amino acids, has also been reported as the cell adhesive biomolecule for the adhesion of neural retina cells.[86]

Negative enrichment of target cells is also an attractive option for separation and enrichment of rare cell types. In this technique the surface of the microchannel is coated with the ligands specific to the non-target cell types in the suspension resulting in adhesion of all cell types except target cells. This method has the advantage of eliminating the difficulty in detaching target cell types from surface after separation. Implementing this technique, adipose-derived stem cells (ADSCs) could be isolated from a suspension of endothelial cells (ECs), smooth muscle cells (SMCs) and fibroblasts (FBs) in a three-stage spiral shape microfluidic platform coated with peptides specific to ECs, SMCs and FBs adhesion (Fig. 7).[76]

Using peptides for specific targeting of cells has not been limited to surfaces. Recently, peptide functionalized hydrogel, namely alginic acid, was used to capture fibroblasts in a microfluidic device.[87] First the alginate was absorbed on the glass surface and then by using Ca^{2+} ionic solution, it was converted to hydrogels. After capturing the target cells by hydrogel, ethylene diamine tetraacetic acid (EDTA) was used to dissolve the alginate and release the cells. It should be noticed that specific cell

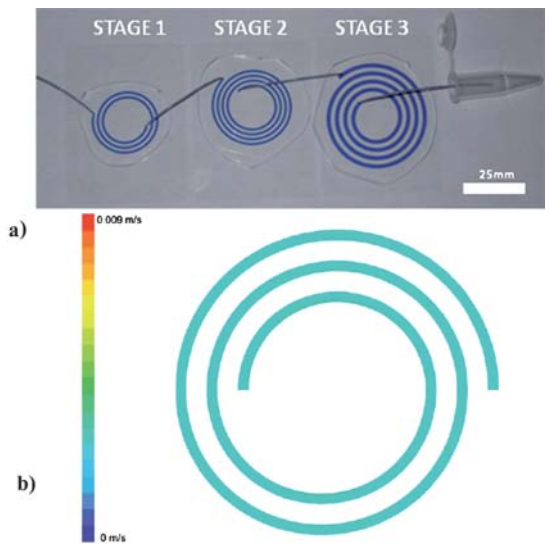

Fig. 7 (a) Experimental set up of the ADSC recovery experiments. From left to right; REDV stage (266 mm^2), VAPG stage (491 mm^2), and the RGDS stage (793 mm^2). (Reproduced from ref. 76 with permission from Royal Society of Chemistry). (b) Velocity profile for a microchannel with spiral geometry obtained by CFD modeling. The inner radius of the spiral is 1.9 mm and the profile shown is uniform throughout the length and cross-section of the channel at all locations. (Reproduced from ref. 70 with permission from Royal Society of Chemistry.)

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capture using gels is a different approach compared to the use of shear stress for cell detachment, in which the cells are released after the gel dissolution. The use of gel for cell entrapment and release might provide an advantage over other cell separation techniques, since it maintains cell integrity.

4.2.3 Aptamer-specific cell adhesion. A recent technique, referred to as Systematic Evolution of Ligands by Exponential Enrichment (SELEX), utilizes RNA, ssDNA or modified nucleic acids as aptamers to selectively target organic molecules, proteins,[88] antibodies[89] or desired cell types.[90–95] SELEX, introduced first by Gold *et al.*[96] and Szostak *et al.*,[97] is an important tool in the diagnosis of diseases as well as in research and therapeutics. Cell SELEX, as an extended development of the SELEX technique, recognizes differences in surface molecule expression between any two types of cells. This technique relies on obtaining a specific probe for a cell type without having any knowledge of molecular differences between cell types.[98] Cell SELEX technique has been used to specifically target human red blood cells,[99] cancer cells[93,100–102] and to separate stem cells.[103]

The cell SELEX procedure includes incubation of the combinatorial RNA or ssDNA library with target cells and the subsequent removal of unbound oligonucleotides (Fig. 8). The bound sequences are then eluted from the cell surfaces and amplified by RT-PCR or PCR for the next SELEX cycles. To overcome unspecific binding of RNA or DNA pools to other sites on cell surfaces other than the desired target site, the preselected RNA or DNA pool is exposed to control cells and only the unbound RNA/DNA fractions are used for the next SELEX cycle. After cycles of target selection and subtraction rounds, a homogenous population of aptamers are found to specifically bind to the target cell.[98]

Finding specific aptamers is an iterative process which requires multiple rounds of selection and amplification. Recently, microfluidic devices have been implemented to discover aptamers[104,105] making the selection process rapid, efficient and automatable. Xinlui *et al.*[105] reported using magnetic bead based SELEX process in a microfluidics device for discovering aptamers.

So far, few groups have implemented aptamers in microfluidic devices for enrichment of cancer cells,[5,106] and detection of biomolecules.[107–109] An interesting aptamer-based microfluidic device for detection of cancer cells was introduced by Xu *et al.*[5] This device selectively captures cells by immobilized DNA-aptamers and yields a 135-fold enrichment of rare cells (Fig. 9). Three different leukemia cell types, CCL-119 T-cell (CCRF-CEM human acute lymphoblastic leukemia), Ramos cells (CRL-1596, B-cell, human Burkitt's lymphoma), and Toledo cells (CRL-2631, non-Hodgkin's B-cell lymphoma) were targeted with this device using three different specific aptamers (sgc8, TD05, Sgd5). This device was reported to target and sort cells with 96% purity.

5. Discussion and future outlook

Microfluidic cell sorting devices have the potential to be used in variety of applications such as preparation of organ transplants,[110] purification of cell mixtures for diagnostic tests such as cancer,[83] umbilical cord blood processing/prenatal blood testing for genetic disorders,[111] tracking tumor cells in the body,[6] stem cell purification for regenerative medicine,[103,112,113] removal of lymphocytes from transfusion for specialized cell packs,[114] cell counting such as white blood cells and CD4 + T cell counting,[115–117] isolation and detection of pathogenic microorganisms and parasites in food, environment and clinical samples[118] and bacterial identification.[21] (Table 3).

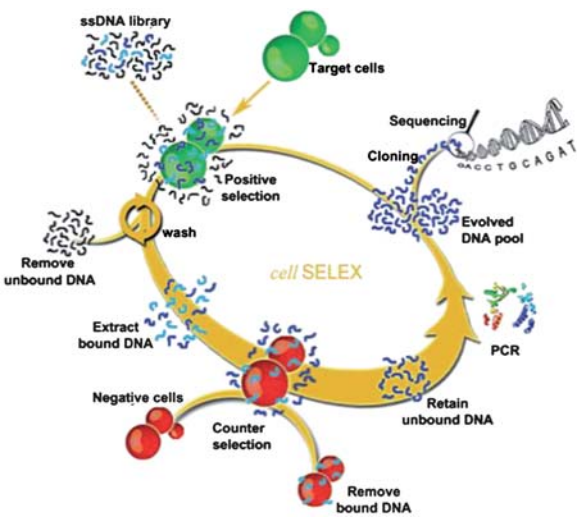

Fig. 8 Schematic representation of the cell-based aptamer selection. The ssDNA pool was incubated with target cells (CCRF-CEM cells in this study). After washing, the bound DNAs were eluted by heating to 95 °C. The eluted DNAs were then incubated with negative cells (Ramos cells in this study) for counter selection. After centrifugation, the supernatant was collected and the selected DNA was amplified by PCR. The PCR products were separated into ssDNA for next-round selection or cloned and sequenced for aptamer identification in the last-round selection. (Reproduced from ref. 92 with permission from Proceedings of the National Academy of Sciences.)

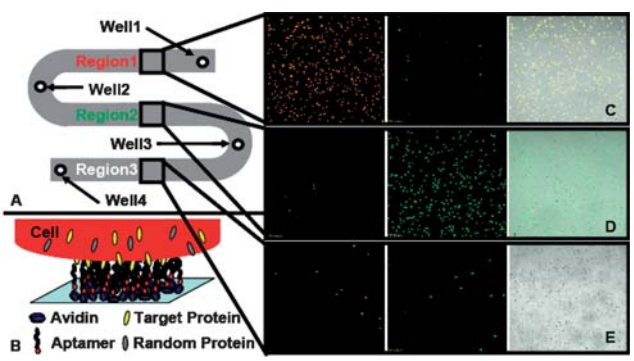

Fig. 9 Microfluidic device and multiplexed detection of 3 different cancer cell lines. (A) Schematic of microfluidic device, showing three regions used for aptamer immobilization and four wells used for channel preparation and cell sample injection. (B) Aptamer immobilization scheme, showing avidin adsorbed to the glass surface and biotinylated aptamers capturing a cell through specific molecular interactions. (C) The Sgc8 aptamer enriches the red-stained CEM cells. (D) The TD05 aptamer enriches the green-stained Ramos cells. (E) The Sgd5 aptamer enriches the unstained Toledo cells. (Reproduced from ref. 5 with permission from American Chemical Society.)

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Table 3 -Applications of cell sorting in biotechnology[7]

General Aim	Sorting Target	Selected Examples
Physiological research, Protein engineering	Viability, vitality, ligand binding	Bacteria, yeast, antibody surface display, peptide surface display
Cellular properties	Enzyme engineering, cell hybridization, cloning, promoter trapping robustness, process related properties	Intra- and extracellular enzymes yeast hybridization, library cloning bacteria, acid tolerance high cell density, low growth rate
Overproduction	Product stained by immunofluorescence autofluorescence of product unspecific staining	Protein, alkaloids, FITC/antibiotic production

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Despite these evident potential applications of cell sorting chips, more investigations are needed to develop microfluidic devices to achieve higher selectivity, less separation time and higher cell capture capabilities. New surface patterning and modification approaches combined with more efficient designs, highly selective cell adhesive ligands and effective detection and separation strategies are the main challenges to overcome and to push the technology from R&D laboratories to the market.

Selectivity can be further enhanced by the discovery of new cell adhesive molecules that are inexpensive and easily applicable on microchannel surfaces. Combining nano-patterned surfaces with subsequent coating of the specific cell adhesion molecules can also help achieve higher and more specific selectivity. This is important in designing a microchannel for separating different cell types at different local points of a microchannel. New designs of micfluidics aiming to achieve optimum shear stress and better positioning of the ligands on the surface showed an enhanced separation time and ratio of captured cells. Parallel separation and sorting using a set of microchannels is often used to increase the capture ratio of cells. The design should allow binding of adhesive molecules for each specific cell type on the areas of the channels in which shear stress is optimum for adhesion of target cell type. Optimum flow rate and shear stress not only depend on the cell type but also on the topography and chemistry of the surface.[62] Furthermore, the geometry of the microchannels and positioning of the ligands should be taken into consideration according to the optimum shear stress to achieve the best adhesion for each cell type. It should be noticed that the shear stress also affects the functionality of cells and cell membrane receptors. This effect has widely been discussed in the literature under the mechanotransduction topic.[119,120] However the mechanotransduction could be used as an approach in conjunction with surface functionalization techniques to enhance the specificity and selectivity of Lab-on-Chip cell sorting devices.

Detachment of adhered cells for subsequent applications is one of the main issues for adhesion-based microfluidic cell sorting devices. On-chip cell lysis is currently used for devices designed for diagnostic applications, but it is not suitable for applications requiring recovery of viable and functional cells.[87] As explained in section 3.3.2 a reliable solution to overcome this problem is negative enrichment where undesired cells are captured, while leaving the target cell line, for instance stem cells, in the flow stream.[76]

Achieving high specificity and selectivity is the key point in optimizing and improving adhesion-based cell sorting devices. Current approaches use biomolecules such as proteins, peptides or aptamers to selectivity capture and separate desired cell types as summarized in Table 4. However, aptamer-based or SELEX technique implemented in microfluidic platforms, remains the reliable and highly specific method, and therefore, the most promising technique for cell research and point of care diagnosis.

Future developments of microfluidic cell sorting devices should combine negative enrichment and aptamer-based techniques to achieve a breakthrough in the field. Negative enrichment will avoid the subsequent detachment of target cells while the use of aptamers will increase the efficiency of separation by capturing target cells with higher selectivity. For high throughput cell separation, the microfluidic platform could be composed of parallel arrays of microchannels, each coated with specific ligands using microfluidic printing. Another promising approach is modifying each microchannel with different ligands to capture more than one cell type in a single channel. Microfluidic printing, microcontact printing or a combination of these two methods can be used for selectively patterning a single microchannel with different ligands. Implementing of parallel channels with the latter will be very time effective and efficient when the separation of a large population of cells is involved.

A new trend to increase the robustness of cell sorting devices, seeks creating artificial ligands or traps on microchannel surfaces that target cell lines through specific site recognition. This approach, so-called molecular imprinting, allows imprinting desired peptides or aptamers on microchannels surfaces to obtain site specific recognition to initially imprinted ligand.[121–123] The use of molecularly imprinted ligands or "artificial ligands", allows for easy, customized production with various template ligands, using common polymers. In addition, imprinted surfaces are selective, robust and applicable under a variety of experimental conditions. They can be mass-produced and therefore the costs associated with the use of biomolecules as templates can be reduced significantly.

As a summary, novel adhesive ligands combined with creative designs will change the trend of adhesion-based cell sorting devices in the future. There is an immediate need to discover and introduce cell specific biomolecules to be used in conjunction with cell separation microfluidic devices. A portable, easy to use and inexpensive adhesion based cell separation microchip can find tremendous applications in early stage diagnosis, blood transfusion and in regenerative medicine for separation of progenitor cells, stem cells and other rare cell types.

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Table 4 Cell adhesion molecules implemented in microfluidic devices

Cell type	Specific ligand	Optimal Shear stress or Flow rate	Specificity and efficiency of separation	References
CCL-119 T-cell (CCRF-CEM human acute lymphoblastic leukemia)	Sgc8 Aptamer	300 nL/s	>85%	5,92
CRL-1596, B-cell, human Burkitt's lymphoma), and TD05,	TD05 Aptamer	300 nL/s	>85%	5,92
Toledo cells (CRL-2631, non-Hodgkin's B-cell lymphoma)	Sgd5 Aptamer	300 nL/s	>85%	5,92
Mesenchymal stem cells (MSCs)	Aptamer G-8	No Flow	85%>	103
Fibroblast	RGDS peptide	1.1 dyn/cm^2	60%–70%	64,76,87
Endothelial cells	REDV peptide fibronectin, Anti-CD34	1.9 dyn/cm^2	60%–90%	80
Smooth muscle cells (SMCs)	VAPG peptide	2.9 dyn/cm^2	83%	63
Neurons	IKVAV Peptide	No flow	—	124,125
Osteoblasts	KRSR peptide	No flow	—	85
B lymphoma WEHI-231	DLWYDAV peptide	No flow	—	29,126
Astrocyte	KHIFSDDSSE peptide	No flow	—	125,127,128
Breast cancer cells	Epithelial membrane antigen (EMA) and epithelial growth factor receptor (EGFR)	15 μL/min for 3 min	30% cancer cells and less than 5% normal cells captured	83
A549 cancer cells	Wheat germ agglutinin (WGA) protein	Stop flow conditions	62% to 74%	66
Cervical cancer cells (HCCC)	α6-integrin (antibody)	20 μL/min (1.3mm/s) for 3 min	Cancer cell capture>30%, Normal cell capture<5%	129
Neutrophils	Anti-CD16b	20 μL/min	—	84
Eosinophils	Anti-eosinophil	20 μL/min	—	84
Lung, prostate, pancreatic, breast and colon cancer circulating cells (CTCs)	Anti-epithelial-cell adhesion-molecule (EpCAM), also known as TACSTD1)	0.4 dyn/cm^2, 1-2mL/h, 460μm/s	50% to 65%	6
CD4 + T lymphocyte	Anti-CD14, optimum concentration: 75 μg/mL	<0.5 dyn/cm^2	> 80%	117
Platelets	Anti-CD36	<0.5 dyn/cm^2	> 80%	117
Type A erythrocytes, Type O erythrocytes	Lectin Helix pomatia, Agglutinin (HpA) or Griffonia simplicifolia I (GSI)	No flow	94%, 95%	130
KG1a (acute myeloid leukemia) cells	Nano-particles coated with P-selectin/Fc Chimera, 5 μg/mL	2 dyn/cm^2	70%	131
Human lymphocyte, MOLT-3	Anti-CD5	0.75 to 1.0 dyn/cm^2	100%, 2.6 min residence time	2
Human lymphocyte cell lines, Raji	Anti-CD19	0.75 to 1.0 dyn/cm^2	75%, 3 min residence time	2
Endothelial progenitor cells, EPC	Anti-CD34, 1010 ligands/mm^2	1.47 dyn/cm^2	54%, 20 fold enrichment	62
Vascular endothelial cells VEC	Anti-CD31	1.47 dyn/cm^2	53%, 28 fold enrichment	62
Leukocytes (white blood cells)	Anti-CD16b	1 μL/min	80%	84
Dendritic cells	Uric acid crystals	No flow	—	132

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