Isolation of circulating tumor cells under hydrodynamic loading using microfluidic technology

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Abstract Cancer is a leading cause of mortality worldwide causing human deaths. Circulating tumor cells (CTCs) are cells that have detached from a primary tumor and circulate in the bloodstream; they may constitute seeds for subsequent growth of additional tumors (metastasis) in different tissues. The detection of CTCs may have important prognostic and therapeutic implications but, because their number

is very small, these cells are not easily detected. Circulating tumor cells are found in the order of 10–100 CTCs per mL of whole blood in patients with metastatic disease. Isolation of tumor cells circulating in the blood stream, by immobilizing them on surfaces functionalized with bio-active coating within microfluidic devices, presents an interdisciplinary challenge requiring expertise in different research areas: cell biology, surface chemistry, fluid mechanics and microsystem technology. We first review the fundamental of cell biology of CTCs and summarize the key microfluidic techniques for isolation of CTCs via cell-ligand interactions, magnetic interactions, filtration; detection and enumeration of CTCs; *in vivo* CTCs imaging.

1 Introduction

Blood is a specialized bodily fluid that delivers necessary substances to every cell in the body, such as nutrients and oxygen, and transports waste products away from those same cells. Blood is circulated around the body through vessels by the pumping action of the heart, and the entire blood volume is re-circulated throughout a human body in about a minute. Blood contains a wealth of information concerning the condition of all tissues and organs in the body. Therefore, analysis of blood samples plays a major role in the diagnosis of many physiologic and pathologic conditions, and extraction of reliable information for clinical or basic research requires the understanding of the relevant biology as well as proper technologies (Toner & Irimia 2005). Indeed, knowledge about blood has expanded with the general progress in biology, while technological advancements enabled several breakthroughs (Wintrobe 1980). Today, staining procedures along with full blood count are still two basic yet very informative blood tests performed in hematology (Bauer 1999). Only flow cytometry can rival these techniques in terms of fine details and high throughput, representing the current state-of-the-art in cell isolation and characterization. The Clinical Laboratory Improvement Act (CLIA), imposing high-quality standards since 1988, has transformed the field of blood analysis (Kost et al. 1999); in particular, analysis of cellular components is restricted to highly specialized laboratories. Nevertheless, despite considerable automation, many blood-handling procedures are still carried out manually or under conditions that could impact the analysis results even in advanced laboratories. Reducing errors and processing time while enhancing analysis capabilities are well-known challenges requiring improved methods.

Microfluidic-based technology is one of the most promising approaches for the next-generation blood analysis (Toner & Irimia 2005). For clinical applications, realizing adequate labs at the patient bedside for comprehensive blood analysis holds the potential of revolutionizing the health-care landscape. Portable systems for reliable blood testing at home or in office will allow accurate and rapid diagnosis of a host of bodily malfunctions such as cancer. They may also accelerate the transition to personalized medicine in effort to reduce side effects and improve the therapeutic efficiency. Microdevices inherently require minute sample volumes for analysis, allowing repetitive sampling while minimizing blood-drawing adverse effects. A diverse array of microfluidic systems have been developed in the last decade for immunoassays including cell sorting, detection and counting, lysis, as well as isolation and amplification of nucleic acids and proteins (Mauk et al. 2007). Microfluidic-

based techniques developed for pathogen detection can be extended for the more challenging task of cancer screening and diagnostics. Automated immunoassays of a single or multiple markers can be implemented for point-of-care cancer screening. More robust tests can be realized by quantifying a panel of nucleic acids or proteins to determine a cancer-specific gene transcription or expression profile. To assess a gene transcription profile, microfluidic systems need to include components for cell sorting and lysis, nucleic acid isolation and amplification, as well as multiplex detection and quantification of gene transcripts. The promising benefits of lab-on-a-chip cancer diagnostics systems include the use of small sample volumes, automated operation, short processing times, reduced reagent consumption, reproducibility and consistency, minimal risk of sample contamination, convenient disposal, and low cost.

This review summarizes recent advancements in the basics of circulating tumor cells (CTCs) and the application of microfluidics for the isolation of metastatic cancer cells circulating in the blood stream. We first present a general overview of disease progression in carcinomas, during which tumor cells from primary tumors of epithelial origin enter blood and lymphatic vessels. This is followed by a brief discussion of the challenges in blood sample preparation and cell separation techniques. Finally, we detail the recent techniques developed for isolation of CTCs, including affinity-based method, magnetic method, filteration, identification and enumeration and imaging method using optical Micro-Electro Mechanical System (MEMS) technology.

## 2 Tumor-derived epithelial cells circulating in the blood stream

Metastasis is the spread of a disease from one organ or part to another non-adjacent organ or part. Only malignant tumor cells and infections have the capacity to metastasize; although, this is recently reconsidered by new research (Cristofanilli et al. 2004, Kahn et al. 2004, Smerage & Hayes 2005). Cancer cells can leave a primary tumor, enter lymphatic and blood vessels, circulate through the bloodstream, and settle down to grow within normal tissues elsewhere in remote sites; and the first observation of circulating tumor cells was described by Ashworth in a post-mortem breast cancer patient (Ashworth 1869). Metastasis is one of the hallmarks of malignancy, and most tumors can metastasize in varying degrees (Zieglschmid et al. 2005). When tumor cells metastasize, a new secondary tumor is formed with its cells resembling those in the original tumor. Viable tumor-derived epithelial cells (CTCs), present in peripheral blood of cancer patients, are most likely the agents of

metastatic disease (Chang et al. 2005, Du et al. 2007, Park et al. 2007, Toner & Irimia 2005).

Epithelial–mesenchymal transition (EMT) occurs during critical phases of embryonic development in many animal species (Kalluri & Weinberg 2009). Without such a transition, in which epithelial are converted into motile cells, multicellular organisms would not be able to advance beyond the early stages of embryonic development. A similar program seems to play a key role in tumor progression as well (Thiery 2002). Epithelial-mesenchymal transition presents a new mechanism for carcinoma progression to more malignant states. Mesenchymal cells develop a morphology during EMT enabling them to migrate in an extracellular domain and to settle in regions associated with organ formation. These cells can be involved also in forming epithelial organs through a reverse mesenchymal–epithelial transition (MET). Although the existence of epithelial and mesenchymal cells was known for over a century, EMT has only recently been identified as a distinct process (Greenburg & Hay 1982). Since then, EMT has become a major research topic (Stoker & Perryman 1985). However, it still took a long time to recognize EMT as an important mechanism driving carcinoma progression. A key reason for this late recognition is due to the inability to follow EMT in space and time in human tumors. The diverse cellular arrangements observed in tumors makes it extremely difficult to perceive EMT with clarity. Nonetheless, some cancer cell lines undergo EMT *in vitro* as illustrated in **Fig. 1**, and pathologists have suggested several criteria for distinguishing between carcinomas and sarcomas (tumors of epithelial and mesenchymal origin, respectively). The mechanisms leading to EMT are now studied extensively; a great similarity between EMT in embryonic development and in tumor progression has been observed, and a few common signaling pathways have been discovered in both EMTs. Thus, the EMT concept provides a new framework for identifying genes important for the cancer progression towards more malignant states.

Loss of E-cadherin expression has been implicated in EMT and, therefore, E-cadherin is proposed as a characteristic of the epithelial phenotype (Holcomb et al. 2002, Yoshida et al. 2001). Renewal expression of E-cadherin in normal and mesenchymal cells can lead to stable contacts among cells resulting in adherens junctions. Initial contacts in epithelial cells are also mediated by E-cadherins that evolve into small complexes to form stable adherens junctions (Adams & Nelson 1998, Garrod et al. 1996, Kowalczyk et al. 1998). While E-cadherin is required for the maintenance of stable junctions, E-cadherin antibodies can break these contacts and induce a mesenchymal phenotype (Imhof et al. 1983). The loss of E-cadherin expression at EMT sites as well as E-cadherin expression at MET sites has been
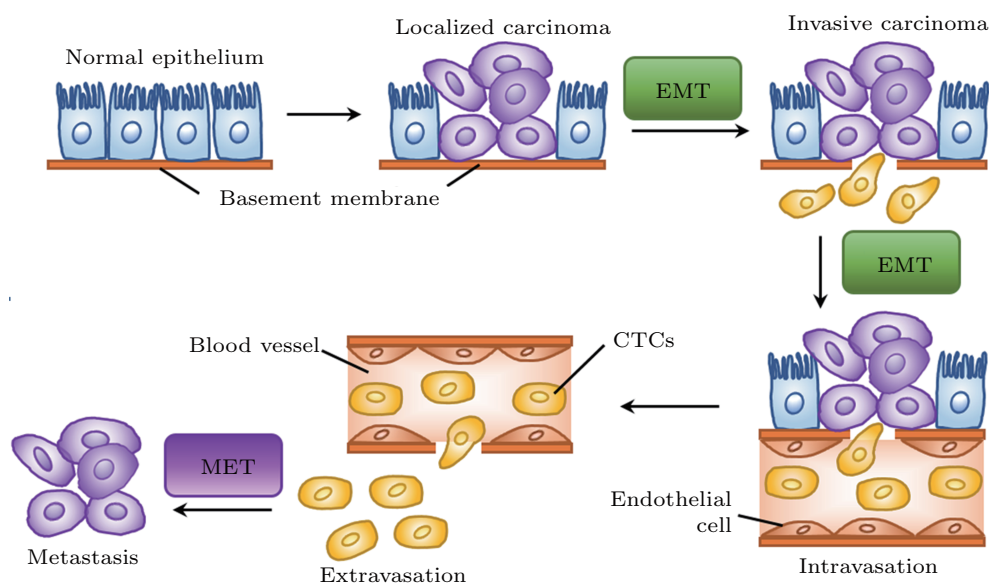
The epithelial-mesenchymal transition (EMT) and mesenchymal-epithelial transition (MET) in cancer progression (modified from Thiery 2002)

documented (Kuure et al. 2000). It has been reported that N-cadherin is expressed in some cancer cells that lost E-cadherin (Frixen et al. 1991). E-cadherin expression is maintained in most differentiated tumors, but E-cadherin levels seem to be inversely correlated with either cancer grade or patient survival (Birchmeier & Behrens 1994, Hirohashi 1998). While contributing to the differentiation-program maintenance, E-cadherin could also important in regulating cell proliferation. Hence, loss of E-cadherin is central to EMT in normal development as well as in cancer cells. The models used for studying EMT *in vitro* suffer from a few drawbacks. EMT is either incomplete or slow in several models, taking several days for completion. Only few cancer cell types of epithelial phenotype can complete EMT *in vitro*. On the other hand, *in vivo*, the molecular mechanisms of EMT have not yet been discerned, although promising results have been obtained in some model systems; EMT in tumors is particularly difficult to study because it can take such a long time. Thus, an important aim of continuing research is to elucidate the precise stages of EMT, both in embryos and in mouse models of carcinogenesis. It might be possible to prevent EMT altogether by understanding the conditions that lead to it. This therapeutic concept could potentially block metastasis and, perhaps, prevent recurrence since micrometastases often survive conventional therapeutic modalities (De Boer et al. 2009).

It is well known that cancer cells can be released from primary tumors and, in animal models, about 10^6 cells/g of tumor ($\sim 10^9$ cells) are estimated to reach the bloodstream each day (Butler & Gullino 1975, Chang et al. 2000). The existence of cancer cells in areas surrounding primary tumors is an indicator of metastasis, and these cells are routinely investigated in various carcinoma types. Circulating tumor cells have also been found in blood of patients having primary tumors, while bone micrometastases have been observed in a large number of patients with tumors smaller than 2 cm in size (Pantel et al. 1999, Thiery 2002). Detection of metastatic tumor cells serves as an independent prognostic indicator for recurrence and survival (Braun et al. 2000, Naume et al. 2001), and could be more significant than lymph-node metastasis in some carcinomas (Braun et al. 2000).

Circulating tumor cells are rare, as few as one cell per 10^9 haematologic cells in the blood of patients with metastatic cancer; hence, their isolation presents a tremendous technical challenge (Cristofanilli et al. 2004, Pantel & Alix-Panabies 2010). However, although extremely rare, CTCs represent a potential alternative to invasive biopsies as a source of tumor tissue for the detection, characterization and monitoring of non-haematologic cancers (Paterlini-Brechot & Benali 2007, Lianidou & Markou 2011). Intensive research effort in this field is now geared towards identifying specific markers (Nelson 2010), and determining the aggressive nature of these cells (Mishra & Verma 2010, Nelson 2010). It is still unknown whether CTCs share similar phenotypic characteristics with cancer cells in either the primary tumor or the lymph nodes, how do they home to specific organs, and how do they flourish in a seemingly hostile environment? Clearly, techniques for the rapid isolation of rare cells from blood would aid in not only diagnostic but also therapeutic applications (Lee et al. 2009).

3 Challenges in cell sorting of blood sample

Blood is composed of two primary constituents: plasma and a variety of cells performing multitude of tasks. The cell mixture in blood is not only very complex but also continuously changing in response to various biochemical and biophysical stimuli; thus, collecting information from blood cells is a major technical challenge. Depending on the required clinical or research information, only a sub-population of cells is relevant and therefore has to be isolated from the blood sample. The other blood cells have to be filtered out as they may interfere with the subsequent analysis of the target cells. However, the sheer number and vast diversity of cell types dramatically complicate the task of selectively isolating specific

cells. In every microliter of blood, there are millions of cells including red and white blood cells (RBCs & WBCs); and, for each WBC, there are about one thousand RBCs in blood. White blood cells (leukocytes) are themselves quite diverse, and their classification into five groups is insufficient for many applications (**Table 1**); a considerable number of subgroups and sub-subgroups that be identified only by the presence of specific proteins, which are either expressed on the surface of or secreted by the white blood cells have been discovered (Goldsby et al. 2003). Several markers are typically needed to identify cells and elucidate their function in the body. This immense cell diversity presents formidable obstacles for obtaining the final desired purity adequate for further analysis. Even if accurately identified, careful extraction of target cells from a complex mixture such as a blood sample is a greater challenge since most WBCs respond rapidly to environmental changes; hence, they can be modified by various procedures during extraction. It has been demonstrated that the exposure of cells to various insults when processing blood samples could alter the original characteristics of the separated cells (Fukuda & Schmid-Schonbein 2002, Hallden et al. 1999, Lundahl et al. 1995); in such a case, further analysis would not be able to recover the *in vivo* state of the target cells.

Lab-on-a-chip technologies offer a promising alternative for overcoming the obstacles associated with the diversity of blood cells and their susceptibility to varying conditions. The new blood processing techniques can be more than just scaling down current assays.

Table 1 Absolute and relative number of cell populations and subpopulations in normal blood (Wintrobe 1980)

Cell type	Average/µL	Percent of WBC	Size
Erythrocytes (RBC)	5 000 000		6-8 and 2 µm
Reticulocytes	30 000–70 000		
Platelets	200 000–500 000		
All leukocytes (total)	5 000–10 000	100%	
Neutrophils	4 000–8 000	40%–66%	10–12
Monocytes	200–800	4%–8%	14–17
Eosinophils	50–300	1%–3%	10–12
Basophils	0–100	0%–1%	12–15
Lymphocytes (total)	1 000–4 000	20%–40%	7–8
CD4 + T Cell	400–1 600	15%–20%	7–8
CD8 + T Cell	200–800	7%–10%	7–8
B-Cell	200–800	8%–12%	7–8
NK	100–500	4%–6%	7–8

Taking advantage of demonstrated concepts for microenvironment as well as single-cell manipulation (Vilkner et al. 2004, Voldman et al. 1999), new techniques have been proposed to overcome the technical obstacles in the analysis of blood. In microfluidic systems, it is possible to efficiently recapitulate the *in vivo* micro-environment of each cell in blood and, thus, minimizing the risk of modifying the original state of the isolated target cells. Novel cell separation principles coupled with their amenability to on-chip integration with other cell-analysis procedures are key elements in the efforts to meet the strict requirements for unbiased analysis of isolated blood cells.

4   Microfluidic-based cell sorting techniques

4.1   Cell-separation microdevices based on mechanical principles

Variations in cell size and shape can readily be observed when probing cell mixtures under a microscope (Wintrobe 1980). These geometrical differences between various cells in blood samples can be utilized in microfluidic devices designed for separation of cells based on mechanically trapping a sub-population of flowing cells. While it is relatively easy to construct such systems using standard micromachining techniques; in general, they do not offer the flexibility to accommodate variations in cell properties within the tested samples. The separation of a selective cell sub-population of a different size would normally require either a different design or a different device with similar design but different dimensions. Microdevices designed based on size-dependent separation frequently also suffer from low efficiency and poor cell purity.

Arrays of pillars and weir-type structures have been employed separating leukocytes from whole blood samples (Wilding et al. 1998). Successive arrays of passages continuously decreasing in size have also been used in separating target tumor cells suspended in normal blood samples (Mohamed et al. 2004). The common feature was based on trapping target and other larger cells at the array, while, allowing smaller cells to pass through some narrow spaces. However, despite their larger size, target cells can also "squeeze" through the same mechanical constrictions compromising the capture efficiency (Wilding et al. 1998). Furthermore, trapping target cells in designated structures can significantly alter the flow pattern within the microdevice; this is a critical aspect when processing blood samples that contain millions of cells. Another interesting phenomenon was observed blood cells were forced to pass through a device featuring an array of coated posts (Carlson et al. 1997). Among the trapped leukocytes, various cells were stopped at different distances along the

array. This self-fractionation of cells was attributed to a combination of differences in viscosity and nonspecific adhesion between the various cell types as they interact with the posts during the separation process. The limited achievements of these early efforts highlight the gaps in our understanding and difficulty in taking advantage of the cell-surface interaction to isolate a selective group of cells.

In an alternative approach, the characteristic motion of particles in a laminar flow has been investigated for separation of different particles. Discrimination of particles varying in size has been demonstrated in microfabricated devices. Particles were separated as a result of a differential lateral movement due to the asymmetry of laminar flow around mechanical obstructions with a length scale on the order of the particles size (Huang et al. 2004). Continuous separation of suspended particles in liquid was also demonstrated by forcing the suspension through a narrow passage with an opening comparable to the particle size. A stream with and a stream without suspended particles were merged together in a pinched microchannel; as a results, the particles were aligned along the sidewall such that smaller particles were closer to the wall, while larger particles were closer to the channel center. Thus, after passing the narrow constriction, initially mixed particles were re-aligned along distinct streamlines depending on their size (Yamada et al. 2004). The flow pattern around a microstep structure was also used for the separation of micro-particles and bacteria (Vankrunkelsven et al. 2004).

The compliance of biological cells varying in size typically compromises the efficiency of size-dependent cell separation; however, some devices were designed to separate cells based on both their size and stiffness. Microchannels simulating blood vessels were used to separate infected and more rigid red blood cells from normal, uninfected ones (Shelby et al. 2003). The higher rigidity of infected cells hinders their passage, resulting in their aggregation at the channel entrance. Separation of plasma and other cellular components from blood samples in microfluidic devices has also been reported. Plasma was collected by driving whole blood through microchannel arrays (Yang et al. 2004, Blattert et al. 2004), or by sample centrifugation in lab-on-a-disk devices (Brenner et al. 2004, Kang et al. 2004).

4.2　Dielectrophoresis-based microdevices for cell separation

In dielectrophoresis (DEP), cells are electrically polarized in a non-uniform electric field to induce affect their dynamic response. In mammalian cells, the induced polarization is determined by numerous physiological conditions (Gascoyne & Vykoukal 2004). The cell response to the applied electric field is sufficiently sensitive to distinguish between different

cell types (Griffith & Cooper 1998). In microdevices, it is rather easy to generate intense electrical fields under low voltages. Moreover, the electric fields can be fine-tuned at a length scale on the order of the cell size by using integrated micro-components. Due to these advantages, many DEP microdevices have been presented for manipulating suspended cells or particles. Separating cells from mixtures in microfluidic devices, using dielectric fields, has been discussed in several reviews (Gascoyne & Vykoukal 2002, Gascoyne & Vykoukal 2004, Hughes 2002), and various strategies have been proposed for dielectrophoretic separation depending on the sample volume.

DEP-based cell trapping is based on creating energy traps with a characteristic scale on the order of the target cell size. Trapped cells are in stable equilibrium; they can be released by turning off or perturbations of the electric field (Voldman et al. 2002, Lapizco-Encinas et al. 2004). The efficiency of capturing target cells can be improved by setting the electric-field frequency such that the non-target cells are driven away (Huang et al. 2002). Under no-flow conditions, the efficiency can be enhanced by dielectric levitation of target cells above electrode arrays (Gascoyne et al. 2002).

Target cells in large samples can be isolated using hyper-layer techniques, in which DEP forces are exerted on cell suspensions driven through microchannels utilizing electrode arrays. The forces acting on each cell include the gravitational force proportional to its density, hydrodynamic drag force proportional to fluid velocity, and DEP force exponentially decaying with its distance from the electrodes. Different cells attain equilibrium states at different channel heights as a result of these three forces. Each cell type then moves with different speed due to the non-uniform velocity profile across the channel. Thus, cells in a flowing mixture were separated along a channel and collected at its outlet at different time intervals (Gascoyne & Vykoukal 2004). The hyper-layer technique was used to separate between normal and cancer cells in whole blood samples (Becker et al. 1995). Interdigitated electrodes were placed at the bottom of thin flow chambers were used to levitate normal and cancerous cells at different heights, depending on their dielectric properties (Yang et al. 1999). However, the separation efficiency has to be improved for clinical applications such as the detection of cancerous cells in clinical blood samples.

DEP forces were also exploited to divert cells into distinct streams, dissociate cell clusters, and separate cells along a particular stream for the isolation of individual cells (Fiedler et al. 1998). Following passage over an interdigitated-electrode array, the suspension main flow was branched into three streams (Holmes et al. 2003); the separation efficiency can be enhanced by forcing the cells into a narrower stream above the electrodes (Holmes

& Morgan 2003). In a different approach, tumor cell lines in mixtures were also separated using DEP forces in devices with integrated microelectrode arrays (Muller et al. 2000, Pethig 1996). Typically, DEP microdevices are simple and do not require pre-treatment of cells; therefore, they are still considered to be prominent candidates for on-chip applications (Yang et al. 2000).

4.3   Microscale optical techniques for cell separation

Optical techniques for separation of cells from complex suspension are very appealing because they do not require intimate contact between the target cells and solid surfaces minimizing the risk of cell activation. Using laser-tweezers in microdevices, single cells can optically be trapped and manipulated. However, although suspended cells can be controlled very precisely, only one cell at a time can be handled (Ozkan et al. 2003). Using such microsystems, manipulation of millions of cells in parallel, as is expected for blood samples in clinical applications, would be extremely challenging. Size-dependent cell separation has been demonstrated using a diode laser bar in a microdevice. Size-based separation of cells can be accomplished as larger cells deviate from their path when crossing a laser beam while smaller cells continue un-affected (Applegate et al. 2004). An adaptive optical lattice, formed as an interference pattern, was utilized for separating certain particles and cells from mixed suspensions based on differences in their size and refractive index (MacDonald et al. 2003). More recently, tunable optical lattices were applied for separating RBCs from WBCs with about 95% efficiency (MacDonald et al. 2004). Such microdevices can be reconfigured easily by adjusting the interference pattern; furthermore, since narrow passages are not required, they are less susceptible to clogging during operation. The wide spread availability coupled and continuous cost reduction of coherent light sources are additional incentives to utilize optical techniques for separating target cells from complex mixtures such as blood samples.

4.4   Magnetic techniques for microscale cell separation

Hemoglobin, the iron-bearing oxygen-transport protein contained in red blood cells only, could provide a clear difference in magnetic characteristics between these and all other blood cells. However, despite the iron content, measurements have shown that RBCs exhibit magnetic behavior similar to that of WBCs, at least in oxygenated blood; a weak paramagnetic behavior was reported for RBCs in deoxygenated blood (Paul et al. 1981). In the presence of high magnetic fields, small differences in paramagnetic characteristics could

be exploited for isolating WBCs from RBCs. Capture of RBCs from whole blood samples was first demonstrated utilizing a metal mesh embedded in a strong magnetic field. The generation of intense magnetic fields still requires very large magnets, which are difficult to miniaturize; nevertheless, it is easier to obtain high magnetic-field gradients in microdevices, and magnetapheresis has been demonstrated experimentally (Fuh et al. 2004, Zborowski et al. 2003). Both particles and cells varying in magnetic characteristics were separated from thin layers of suspensions flowing under an externally applied magnetic field (Fuh et al. 2004).

The performance of magnetic-based cell separation can dramatically be enhanced by binding magnetic beads decorated with ligands to membrane receptors on the surface of the target cells. However, in this approach, the cell identification is based on protein: protein specific interaction rather than on differences in cellular magnetic properties. Therefore, microdevices employing this principle are considered to be part of the affinity-based separation class.

4.5   Biochemical interactions for cell separation in microfluidic devices

Subtle biochemical variations between cell populations can be exploited effectively for manipulation of selective cell sub-populations by exposing the entire cell suspension to particular environmental conditions. For example, WBCs are much less vulnerable to ammonium chloride than RBCs which, under exposure to such a solution, are lysed within a very short time period (Szilard 1923). In standard protocols, blood samples are mixed with the lysing-agent solutions for time intervals sufficiently long to allow RBC lysis. However, WBCs are also exposed at the same time to the same agent and, therefore, could be affected to a certain degree by the lysing solution. As a result, it is highly desirable to shorten the exposure time in order to minimize the risk of damaging the WBCs (Kouoh et al. 2000). Experiments and simulations suggest that the diffusion of the lysing agent within the cell suspension is the limiting factor in trying to speed up the reaction; hence, shortening the distance required for diffusion would provide a significant advantage. In microfluidic devices, short diffusion length and time scales are inherent system characteristics. Indeed, lysis of RBCs has been completed within one minute of sample exposure to an isotonic lysis buffer with recovery of almost all the WBCs. In hypotonic deionized water, lysis of RBCs was completed even faster while the WBCs remained intact (Sethu et al. 2004). Cell lysis in microfluidic devices clearly results in improved WBC yield and viability due to the shorter processing time. Furthermore, all cells in the sample are subjected to the same treatment; hence, by eliminating

another source of variability, subsequent analysis could be more reliable.

5   Antibody-mediated isolation of CTCs

Cellular adhesion is the binding of a cell to another cell or to a surface or matrix, and it is regulated by specific cell adhesion molecules that interact with molecules on the opposing cell or surface. Such adhesion molecules are also termed "receptors" and the molecules they recognize are termed "ligands" (or "counter-receptors"). Cells are not often found in isolation, rather they tend to stick to other cells or non-cellular components of their environment. Therefore, a major research effort has been devoted to discover what makes cells sticky. Cell adhesion molecules (CAMs) are proteins located on the cell surface involved in binding with other cells or with extracellular matrix (ECM). These proteins are typically transmembrane receptors composed of three domains: an intracellular domain that interacts with the cytoskeleton, a transmembrane domain, and an extracellular domain that interacts either with other CAMs of the same kind (homophilic binding) or with other CAMs or the extracellular matrix (heterophilic binding). Most of the CAMs are classified in 4 protein families: Ig (immunoglobulin) superfamily (IgSF CAMs), integrins, cadherins, and selectins.

Immunoglobulin superfamily CAMs (IgSF CAMs) are either homophilic or heterophilic and bind to integrins or different IgSF CAMs. Some molecules included in this family are: NCAMs Neural Cell Adhesion Molecules, ICAM-1 Intercellular Cell Adhesion Molecule, VCAM-1 Vascular Cell Adhesion Molecule, PECAM-1 Platelet-endothelial Cell Adhesion Molecule, L1, CHL1, MAG, nectins and nectin-like molecules. Integrins are a family of heterophilic CAMs that bind to IgSF CAMs or the extracellular matrix. They are heterodimers, consisting of two noncovalently-linked subunits, called alpha and beta. Twenty-four different alpha subunits that can link in many different combinations with the 9 different beta subunits are known; however, not all combinations are observed. Cadherins are a family of Ca^{2+}-dependent homophilic CAMs. The most important members of this family are E-cadherins (epithelial), P-cadherins (placental), and N-cadherins (neural). Selectins are a family of calcium-dependent heterophilic CAMs that bind to fucosylated carbohydrates, e.g., mucins. The three family members are E-selectin (endothelial), L-selectin (leukocyte), and P-selectin (platelet). The best-characterized ligand for the three selectins is P-selectin glycoprotein ligand-1 (PSGL-1), which is a mucin-type glycoprotein expressed on all white blood cells.

6   Microfluidic devices for the isolation of CTCs

The analysis of circulating tumor cells (CTCs) includes isolation, characterization and enumeration of CTCs. CTCs are very rare (as low as few CTCs per 10 mL of blood), the isolation of CTCs is therefore technologically challenging. Ideally, a platform for capturing CTCs should have the following characteristics: (i) high sensitivity, i.e., detection of every single target CTC (no false negative); (ii) high specificity, i.e., removal of all non-target cells (no false positives); (iii) high purity, i.e., isolation of all target CTCs with no contaminating non-target cells; (iv) preservation of CTC viability, morphology, protein and nucleic acids; rapid and minimal processing of blood could improve the quality of CTC material for molecular characterization studies; and (v) economical, i.e., low cost, high-throughput and minimal operation time.

In the absence of any gold standard with which to evaluate various technologies, defining their absolute accuracy, sensitivity, and specificity in detecting CTCs remains a challenge. The ultimate goal, i.e., to efficiently isolate this rare sub-population of cells in a viable and intact state and with high purity from the vast number of surrounding blood cells, presents a daunting technological challenge. In recent years, substantial progress has been made to improve and automate the isolation and characterization of CTCs, increasing the detection sensitivity while decreasing the cost. These innovative CTC analytical technologies hold the potential to serve as highly efficient and low-cost diagnostic tools for cancer. In general, there are two basic formats that can be used for CTC isolation: macroscale and microscale.

Commonly used macroscale systems for CTCs detection include: (a) magnetic tagging utilizing ferromagnetic micro-beads functionalized with homing ligands, classified as immunomagnetic-assisted cell sorting , such as CellSearch[TM] (Allard et al. 2004); (b) size-based separations that use porous membranes, such as ISET (Paterlini-Brechot & Benali 2007, Vona et al. 2000), ScreenCell[TM] (Vona et al. 2000); (c) fluorescence-activated cell sorting (FACS) (Wang et al. 2012); and (d) reverse-transcription polymerase chain reaction (RT-PCR) of mRNAs (AdnaTest BreastCancer[TM]) (Lankiewicz et al. 2006), which are used as surrogates for cell identification. In general, commercial systems allow the collection of rare cells from large sample volumes; however, their operation involves overcoming serious obstacles that include labor-intensive sample preparation, sample loss due to transfer between instruments, and high cost associated with the completed device as well as the large quantity of reagent consumption.

In comparison, microfluidic systems enable the application of processing techniques

which are difficult to utilize in macrosystems (Chen et al. 2012, Das & Chakraborty 2013, Dong et al. 2013, Sequist et al. 2009). In the microscale domain, the behavior of the fluid is more predictable. Therefore, it is possible to take advantage of micro and nanostructures to improve the capture efficiency of viable rare cells. Particularly, microsystems allow handling individual cells without loss, which is not easy to duplicate with conventional systems. Microsystem technologies are also attractive due to the smaller physical dimensions, reduced power requirements, lower reagent consumption and high-throughput automation potential in comparison with macroscale alternatives. Furthermore, CTC characterization following capture can also be integrated into a microsystem to provide a fully integrated analysis. A comparison of the fabrication technology, blood sample requirements, capture efficiency, target cancer cells, cell viability, clinical assessment, required processing time of various micro/nano CTC chips is summarized in **Table 2**. In addition, the detailed performance of the systems for the detection of CTC developed by private companies is summarized in **Table 3**.

6.1　CTC isolation via cell-ligand interactions

Affinity-based isolation of CTCs is frequently utilized in microfluidic systems. This method relies on the specific interaction between a ligand — an antibody (Nagrath et al. 2007, Santana et al. 2011), aptamer (Dharmasiri et al. 2009, Fan et al. 2009), or lectin (Wang et al. 2010), and a particular receptor on the surface of the target cell. Affinity-based cell isolation can be achieved by either positive or negative approach. Negative method aims to remove all non-target cells with minimal capture of target cells; this method is attractive when the target-cell biomarkers are not well known. Positive method aims to capture directly the target cells by exploiting surface markers unique to a certain cell sub-population. Typically, cell selection requires a fluidic conduit bounded by a solid surface on which the homing ligands are immobilized, and the CTC-containing sample is forced through this conduit to enable the selective binding interaction.

Essential factors to achieve high-performance CTC isolation are: (a) frequency of contact between target cells and the chemically modified solid phase, (b) adhesion strength between target cell and recognition ligands, (c) specificity of cell receptor-surface ligand interactions, (d) adequate flow rate to maintain the stability of cell-ligand binding, and (e) high throughput. In microfluidic devices, microchannels may include additional micro or nano features designed to enhance the cell-surface interactions (Isselbacher et al. 2010, Wang et al. 2011). By applying properly configured topography of the solid phase (Talasaz

Table 2 Summary of various micro/nano CTC chips

References	Device name	Operation principle	Sample Test		Clinical Assessment		Time Consumption	Fabrication complexity[a]	Overall score[b]
			Cell Lines; Medium; Volume/mL; Optimum throughput/(mL·h⁻¹)	Capture efficiency; Purity; viability	Cancer type; Sample volume/mL	Sensitivity; Specificity	Sample processing; Turnaround /min		
Nagrath et al. 2007	Micropost chip	Antibody coated microposts	PC3-9, SkBr-3, T-24, NCI-H1650; PBS, WB; NA; 1–2	>65%; 50%; ~99%	Lung, Prostate, Pancreas, Breast, Colon; 2.7	99%; 100%	81–162; 398–472	2	3
Zheng S et al. 2007 Lin H K et al. 2010	Microfilter	Size based filtering	LNCaP; PBS, WB; 1; 6	>90%; NA; ⩾ 90%	Prostate, Breast, Colon, Bladder; 7.5	96% 16%	<10; 150	1	4
Adams et al. 2008 Soper et al. 2009 Soper et al. 2011	HTMSU chip	Antibody/aptamer coated curvilinear microchannels	MCF-7, LNCaP, SW620, HT29; WRB, WB; 1; 2	90%– 97%; 100%; NA	NA	NA	29–40; 279–290	2	2
Fan et al. 2009 Sheng et al. 2012	Micropillar chip	Aptamer coated micropillar	DLD-1, HCT 116, CCRF-CEM; PBS, WB; 1; 2.16	~90%; ~81%; ~93%	NA	NA	28; ~250	2	3
Lim C T et al. 2009 Tan et al. 2010	Micro crescent well chip	Size and deformability based filtering	MCF-7, MDA-MB-231, HT-29; PBS, WB; 1–3; 0.7	>80%; >83%; NA	NA	NA	86; ~450	1	2
Talasaz et al. 2009	MagSweeper	Antibody coated magnetic beads	MCF-7; PBS, WB; 9; 9	>50%; 51%– 100%; 94%	Breast; 9	100%; NA	60; >130	1	4

Table 2 Summary of various micro/nano CTC chips (continued)

References	Device name	Operation principle	Sample Test: Cell Lines; Medium; Volume/mL; Optimum throughput/(mL·h⁻¹)	Sample Test: Capture efficiency; Purity; viability	Clinical Assessment: Cancer type; Sample volume/mL	Clinical Assessment: Sensitivity; Specificity	Time Consumption: Sample processing; Turnaround /min	Fabrication complexity[a]	Overall score[b]
Wang S et al. 2009	SiNP chip	Antibody coated SiNP	MCF-7, PC3, HeLa, Daudi; PBS, WRB; 1; NA	45%–65%; NA; 84%–91%	NA	NA	45; 205	2	2
Gleghorn et al. 2010; Kirby et al. 2012	GEDI chip	Antibody coated staggered obstacle arrays	LNCaP; PBS, WB; 1;1	85%–97%; 62%–68%; NA	Prostate; 1	94%; NA	60; >520	2	3
Hosokawa et al. 2010; Hosokawa et al. 2013a; Hosokawa et al. 2013b	Microcavity array	Size based filtering	NCI-H358, MCF-7, SW620, AGS, SNU-1, Hs578T, NCI-H69, NCI-H82, NCI-H358, NCIH441, HCC827, DMS79, A549, PC-14; PBS, WB; 1; 12	80%–99%; ~33%; 98%;	Lung; 4	95.7%; 20%	20; 120–180	1	4
Isselbacher et al. 2010	Herringbone chip	Herringbone-Antibody and herringbone mixer	PC3; PBS, WB; NA; 2–3	92%; 14%; 95%	Prostate; 4	93%; NA	100–160; 300–400	2	3
Lu B et al. 2010; Xu et al. 2010	Microslot filter	Size based filtering	PC3, DU145; WB; 1; 12	90%; NA; 90%;	Prostate; 7.5	NA; 93%	38; NA	1	4
Wang J Y et al. 2010	Lectin-aided chip	Microcolumn and lectin	K562; WMB; NA; 0.06	84%; 99%; 94%	NA	NA	NA; >168	2	3

Table 2 Summary of various micro/nano CTC chips (continued)

References	Device name	Operation principle	Sample Test: Cell Lines; Medium; Volume/mL; Optimum throughput/(mL·h⁻¹)	Capture efficiency; Purity; viability	Clinical Assessment: Cancer type; Sample volume/mL	Sensitivity; Specificity	Time Consumption: Sample processing; Turnaround /min	Fabrication complexity[a]	Overall score[b]
Di Carlo et al. 2010	Inertial microfluidics	Deformability induced inertial force	Hela, MCF7; WB; 1; 0.17	97%; NA; >91%	NA	NA	360; >380	1	2
Alazzam et al. 2011	Comb-like DEP chip	DEP	MDA231; WB; NA; 0.1	96%; NA; NA	NA	NA	<60; NA	1	1
Jen et al. 2011 Jen et al. 2012	DEP-FFF chip	DEP	Hela; WB; NA; NA	76%; NA; 100%	NA	NA	~3; NA	1	1
Di Carlo et al. 2011 Hur et al. 2011	Microvortice chip	Size induced inertial force	Hela, MCF7; WB; 1 and 10; 300	10%–20%; 85%; 90%	NA	NA	<3; <63	1	3
Duraiswamy et al. 2011	SiNP chip	Antibody coated SiNP with micromixers	MCF7; PBS, WB; 1; 1	>95%; NA; NA	Prostate; 1	75%; 22%	60; >550	3	2
Hoshino et al. 2011	Immuno-magnetic chip	Magnetic nanoparticles conjugated to antibody	COLO205, SKBR3; WB; 3.5; 10	90%; NA; NA	NA	NA	21; >75	1	2
Jung et al. 2011	MOFF-DEP chip	Combine multi-orifice flow fractionation (MOFF) with DEP	MCF7; WB; 2; 7.56	99%; 16%; NA	NA	NA	16; NA	1	2

Table 2 Summary of various micro/nano CTC chips (continued)

| References | Device name | Operation principle | Sample Test | | Clinical Assessment | | Time Consumption | Fabrication complexity[a] | Overall score[b] |
			Cell Lines; Medium; Volume/mL; Optimum throughput/(mL·h^{-1})	Capture efficiency; Purity; viability	Cancer type; Sample volume/mL	Sensitivity; Specificity	Sample processing; Turnaround /min		
Bhagat et al. 2011	Pinched flow coupled shear-modulated inertial microfluidics	Size based inertial force	MCF-7, MDA-MB-231; PBS, WB; 1; 24	>80%; NA; NA	NA	NA	~50; >145	1	2
Thierry et al. 2010 Thierry et al. 2011	MicroPDMS chip	Antibody coated PDMS microposts	SK-BR-3, NCI-H69; PBS, WB; −0.5; 1	>70%; NA; NA	NA	NA	30; >1500	2	1
Wo et al. 2011 Chen et al. 2012	Microdisk chip	Magnetic and centrifugal force	MCF-7; PBS; 0.2; −1.7	80%; NA; 90%	NA	NA	~7; >85	2	3
Zheng S et al. 2011	3D micro-filter	Size based filtering with 2 layers of membranes	LNCaP, MCF-7; WB; 10; 120	~86%; NA; >85%	NA	NA	3–5; ~50	2	3
Zheng X et al. 2011	High-performance microsystem	Antibody coated microchannels	MDA-MB-231; PBS; 0.02; 0.03	95%; 85%; NA	NA	NA	40; >70	2	3
Augustsson et al. 2012	Acoustophoresis chip	Ultrasonic resonances force	DU145, PC3, LNCaP; PBS, WB; 1; 4.2	93.9%; 99.7%; ~100%	NA	NA	14; >60	1	4

Table 2 Summary of various micro/nano CTC chips (continued)

| References | Device name | Operation principle | Sample Test | | Clinical Assessment | | Time Consumption | Fabrication complexity[a] | Overall score[b] |
			Cell Lines; Volume/mL; Medium; Optimum throughput/(mL·h^{-1})	Capture efficiency; Purity; viability	Cancer type; Sample volume/mL	Sensitivity; Specificity	Sample processing; Turnaround /min		
Bichsel et al. 2012	CTC capture and 3D culture platform	Antibody coated micropillars	PC3; WB; NA; 1	8%–42%; NA; 52%	NA	NA	NA	2	0
Issadore et al. 2012	µHD chip	Antibody coated magnetic nanoparticles and micro-Hall detector	A431, MDAMB-468, MDA-MB-453, SkMG3; NA; NA; 10^7 cells/min	NA	Ovarian; 7.5	91%; 100%	120; 150	3	2
Kang et al. 2012	Microfluidic-magnetic chip	Antibody coated magnetic beads	M6C; WMB; 0.1; 1.2	~87%; NA; 90%	NA	NA	5; >45	2	3
Schiro et al. 2012	MicroeDAR chip	Fluorescent antibodies	SKBr-3, MCF-7; WB; 1; 3	93%; 10%–50%; NA	Breast; 1–2	100%; 0	20; >230	1	3
Lim L S et al. 2012	Microsieve chip	Size based filtering	HepG2, MCF-7, BT474; WB; 1; 60	>80%;–1; NA	Breast; 3	NA	3; <90	1	3
Sun et al. 2012	Double microspiral chip	Size-dependent hydrodynamic forces	Hela, MCF7; WB; 1; 20	97%; NA; NA	NA	NA	3; NA	1	2

Table 2　Summary of various micro/nano CTC chips (continued)

References	Device name	Operation principle	Sample Test		Clinical Assessment		Time Consumption	Fabrication complexity[a]	Overall score[b]
			Cell Lines; Volume/mL; throughput/(mL·h⁻¹) Medium; Optimum	Capture efficiency; Purity; viability	Cancer type; Sample volume/mL	Sensitivity; Specificity	Sample processing; Turnaround /min		
Loutherback et al. 2012	MicroDLD array	Size-dependent deterministic lateral displacement	MCF10A, 231; WB; 5; 600	>85%; NA; >95%	NA	NA	0.5; NA	1	3
Hou et al. 2013	DFF chip	Size-dependent centrifugal forces	MCF-7; WB; 1; 3	85%; ~10%; >98%	Lung; 7	100%; NA	120; >150	1	4
Lin M X et al. 2013	Microvortex chip	Antibody coated magnetic beads and size based filtering	MCF-7, MDA-MB-231; WB; 7.5; 36	91.3%; NA; NA	NA	NA	11.25; >60	2	2
Hyun et al. 2013	p-MOFF chip	Size-dependent inertial forces	MCF-7, MDA-MB-231; WB; 10; 36	>91%; NA; NA	Breast; 7.5	19 out of 24 patients	< 30; <60	1	3
Lu Y T et al. 2013	NanoVelcro chip	Antibody coated SiNW and PDMS chaotic mixer	PC3, LNCaP, C4-2; PBS, WB; 1; 0.5	>80%; NA; NA	Prostate; 1	NA	2; >240	3	2
Huang et al. 2013	ODEP chip	Optically induced-DEP	PC3, OEC-M1; WB; 0.001; 0.006	61%; 64%; 92%	NA	NA	10; NA	2	2

Table 2 Summary of various micro/nano CTC chips (continued)

References	Device name	Operation principle	Sample Test		Clinical Assessment		Time Consumption	Fabrication complexity[a]	Overall score[b]
			Cell Lines; Medium; Optimum throughput/(mL·h^{-1})	Capture efficiency; Purity; viability	Cancer type; Sample volume/mL	Sensitivity; Specificity	Sample processing; Turnaround /min		
Warkiani et al. 2014	Slanted microspiral chip	Size-dependent inertial lift forces	MCF-7, T24, MDA-MB-231; WB; 7.5; 102	~85%; ~4log depletion of WBCs; >90%	Breast, Lung; 7.5	10 out of 10 patients	< 8; ~60	1	5

Abbreviations: PBS, phosphate buffered saline; WB, whole (human) blood; WMB, whole mouse blood; WRB, whole rabbit blood; DEP, dielectrophoresis; MOFF, multi-orifice flow fractionation; FFF, field flow fractionation; DLD, deterministic lateral displacement; DFF, dean flow fractionation; SiNP, silicon nanopillar; SiNW, silicon nanowire; NA, not available. ^{a} The score of fabrication complexity +1 for (1) every mask layer, (2) the special structure in the chamber (with another mask) and (3) antibody coating. ^{b} The overall score (0~5) +1 if the device has a (1) relatively high capture efficiency of >80%, (2) purity of >50%, (3) viability of >90%, (4) throughput of >5 mL/hr, (5) throughput of >5 mL/hr as well as an acceptable turnaround time of <3 hr, and (6) clinical assessment that shows its potential in clinical use.

Table 3 Summary of the CTC system developed by the companies in the world

Platform	Company	Operation principle	Turnaround time/min; Blood sample/mL	Pros	Cons	Clinical applications	References
CellSearch™	Veridex, LLC, Raritan, NJ, USA	Immunomagnetic enrichment and immunofluorescence	~90; 7.5	FDA approved; Validated in multiple clinical trails	High complexity; Captured CTCs are no longer alive	Metastatic breast, prostate and colorectal cancer	Cristofanilli et al. 2004
AdnaGen™ CTC Detection	Adnagen AG, Langenhagen, Germany	Immunomagnetic enrichment and RT-PCR	~480; 5	CE marked; Providing genetic profiles	Low automaticity; without CTC counting	Metastatic breast, and colorectal cancer	Fehm et al. 2009
CTCscope™	Advanced Cell Diagnostic, Hayward, CA, USA	RNA in situ hybridization (ISH) and immunofluorescence	>300; 5	Without the need for special CTC enrichment or purification	Can't capture the apoptotic or dead CTCs	Simultaneous identification and molecular characterization of CTCs	Payne et al. 2012
OncoCEE-BR™	Biocept Inc., San Diego, CA, USA	Immunocytochemical microfluidic device with HER2 FISH	420–540; 16	Capture CTCs undergoing EMT	Large volume blood sample required	CTC enumeration with CK detection or HER2 FISH	Mayer et al. 2011; Pecot et al. 2011
HD- CTC™	Epic Sciences, La Jolla, CA, USA	Immunofluorescence and morphological identification	NA; NA	Without the need for CTC isolation	With CTC cluster problem	Advanced prostate, breast, lung, and pancreatic cancer.	Wendel et al. 2012
Halloysite Nanotubes (HNT™)	NaturalNano Inc., Rochester, NY, USA	Immunocytochemical microtube	>240; 10	Straightforward technique using off-the-shelf materials	Low automaticity	CTC isolation	Hughes et al. 2012

Table 3 Summary of the CTC system developed by the companies in the world (continued)

| Platform	Company	Operation principle	Turnaround time/min; Blood sample/mL	Pros	Cons	Clinical applications	References
Vita-Cap™, Vita-Assay™	Vitatex Inc., Stony Brook, NY, USA	Cell Adhesion Matrix(CAM)	NA; NA	Not biased to capture by particular physical or antibody markers	Only capture invasive CTCs	iCTC capture and culture	www.vitatex.com
Rarecells® System: Patented ISET	RareCells SAS, Paris, France.	CTC size	~120; 10	CE-IVD labeled; Without a previous immune-based selection	Low purity and loss of CTCs smaller than 8 μm size	CTC isolation (except Chronic Lymphocytic Leukemia, CLL)	www.rarecells.com; Farace et al. 2011
ApoStream™	ApoCell, Houston, TX, USA	Dielectrophoresis field flow fractionation (DEP-FFF).	NA; 0.05–10	Antibody-independent; High automaticity	Limited sample suspension collection volume	CTC isolation	www.apocell.com
Canopus' CTC Platform	Canopus Bioscience ltd., Toronto, Canada	Viscoelastic properties	NA; NA	Free from filter clogging	With a first stage removes red blood cells	CTC isolation	http://canopus-bioscience.com/
CellSieve™	Creatv MicroTech, Inc. MD, USA	CTC size	NA; 10	Rapid sample filtering (<2 min)	With CTC cluster problem	CTC isolation, enumeration, and culture	www.creatvmicro-tech.com
ClearCell®FX System, CTChip®FR	Clearbridge BioMedics Pte Ltd., Singapore	Inertial focusing principle	>150; ~6	Entirely label-free	Low purity	CTC isolation	www.clearbridge-biomedics.com; Hou et al. 2013

Table 3　Summary of the CTC system developed by the companies in the world (continued)

Platform	Company	Operation principle	Turnaround time/min; Blood sample/mL	Pros	Cons	Clinical applications	References
ClearCell®CX, CTChip®CS, CTChip®CR.	Clearbridge BioMedics Pte Ltd., Singapore	CTC size and deformability	~450; ~3	Label-free isolation	High complex system	Integrated system for CTC enumeration, staining and retrieval	www.clearbridge-biomedics.com; Lim C T et al. 2009
sureCELL®	CellSievo Pte Ltd., Singapore	CTC size	~90; 3	Label-free isolation	Low purity	CTC isolation and enumeration	Lim L S et al. 2012
Cynvenio's Platform	Cynvenio Biosystems, Westlake Village, CA, USA	Immunomagnetic beads	>150; 5	Isolation via ultrahigh magnetic gradient	Only capture EpCAM+/CK+ CTCs	CTC isolation	www.cynvenio.com
OncoQuick®	Greiner Bio-One GmbH, Frickenhausen, Germany	Buoyant density	>45; 15–30	Fast, antibody-independent	Large volume blood sample required	CTC isolation	www.greinerbioone.com
ScreenCell®	ScreenCell, Paris, France	CTC size	3; 1	Independent of any equipment; CE-IVD labeled	Low purity	CTC isolation	www.screencell.com; Desitter et al. 2011

et al. 2006, Wang et al. 2011, Wang et al. 2009), it is possible to achieve high adhesion strength between target cells and functionalized surfaces. Highly specific recognition ligands, such as monoclonal antibodies and aptamers, are immobilized on the capture surface with a proper density to result in stable and selective binding.

Nagrath et al. (2007) developed a microfluidic system for removing CTCs from whole blood samples (**Fig. 2(a)**). The CTCs originated from various solid tumors and were targeted using epithelial cellular adhesion molecule (EpCAM) monoclonal antibodies immobilized on the surfaces of a microchannel and micropillars. The device contained 78 000 microposts that were 100 µm tall and 100 µm wide with a total surface area of 970 mm^2. The EpCAM antibodies provided the selectivity for the capture of CTCs from blood samples since EpCAM receptors are expected to be overexpressed in epithelial originated cancers (Baeuerle & Gires 2007, Went et al. 2004). Under a 1 mL/h flow rate, 65% of the target CTCs were recovered, while 98% of the captured cells were viable. Enrichments from blood samples yielded about 50% purity. A similar system was also used for isolating rare lung cancer cells from blood samples (Maheswaran et al. 2008). Utilizing the CTC chip, CTCs were detected in 100% of early-stage prostate cancer patients. The application of the chip for monitoring patient reaction to anticancer therapy was also tested. In a group of patients with metastatic cancer undergoing systemic treatment, variations in the number of CTCs were found to correlate with the disease clinical course (Maheswaran et al. 2008, Nagrath et al. 2007).

High CTC capture efficiency is expected due to the enhanced interaction between the target CTC receptors and the immobilized surface ligands. However, fluid flow in microchannels is laminar in nature and, consequently, cells follow streamlines and display minimal species diffusion across flow channels. This lack of mixing results in a limited number of receptor-ligand encounters, which is critical for target cell capture. Stotta et al. (Isselbacher et al. 2010) developed a CTC capture microdevice (**Fig. 2(b)**), using an alternative strategy that involves the use of surface ridges or herringbones in the wall of the device to disrupt streamlines, maximizing contact between target cells and the antibody-functionalized walls. The herringbone-chip (HB-Chip) provided an enhanced platform for CTC isolation. Efficient cell capture was validated using defined number of cancer cells spiked into a control blood sample, and clinical utility was demonstrated in specimens from patients with prostate cancer. CTCs were detected in 93% of the (14/15) patients with metastatic disease. The HB-Chip proved to be highly efficient; at a flow rate of 1.2 mL/h, the capture efficiency of PC3 cells was 79%.
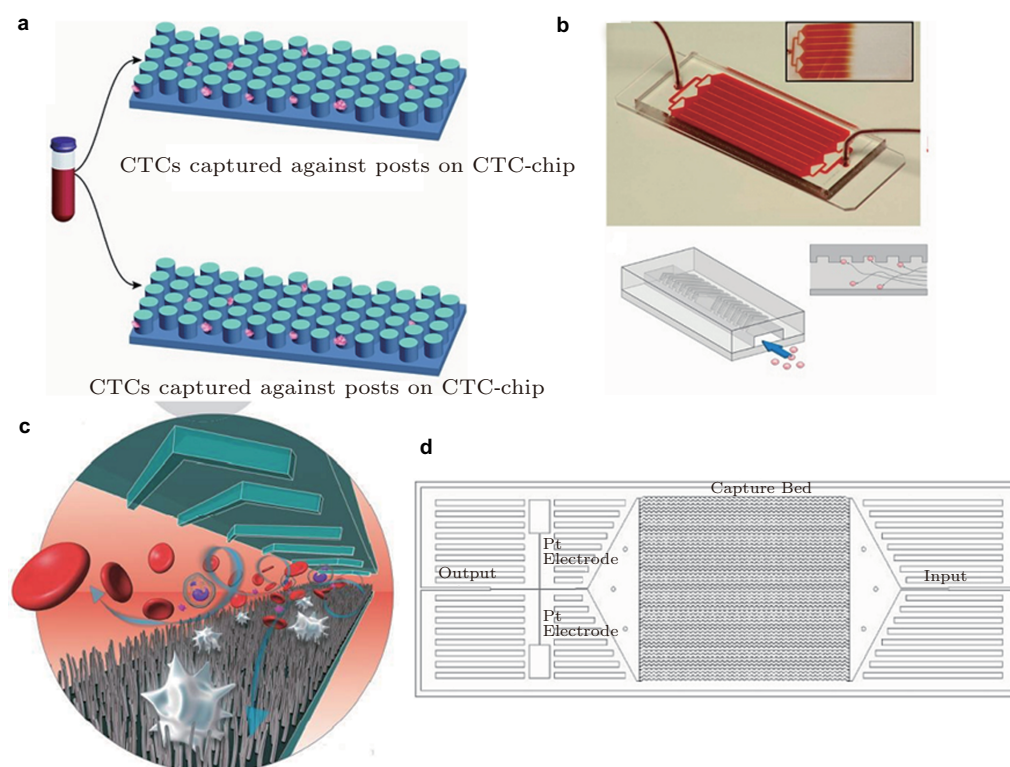
Fig. 2

Representative micro/nano devices for CTC isolation via cell-ligand interactions. (a) Schematic showing CTCs captured by anti-EpCAM coated microfluidic channel and posts. (Nagrath et al. 2007). (b) Cartoon illustrating the cell-surface interactions in the Herringbone-Chip (Isselbacher et al. 2010). (c) Schematic of a microfluidic device featuring an anti-EpCAM coated silicon nanopillar substrate and herringbone-patterned ceiling to constantly bring CTCs into contact with the substrate. (Wang et al. 2011). (d) Schematic of a microfluidic device with integrated systems for CTC capture, enumeration, and electro-manipulation. (Dharmasiri et al. 2009)

An anti-EpCAM-functionalized, nanostructured substrate was developed for the isolation of CTCs from whole-blood samples, **Fig. 2(c)** (Wang et al. 2009, 2011). The uniqueness of this approach lies in the use of 3D nanostructured substrates—specifically, a silicon-nanopillar (SiNP) array—which allow for locally enhanced interactions between the SiNP substrates and nanoscale components of the cellular surface (e.g., microvilli and filopodia) leading to vastly improved cell-capture affinity compared to unstructured flat substrates (Wang et al. 2009). This work showed that the number of captured EpCAM-positive cells increased with increasing SiNP lengths, but relatively minor changes were observed for

EpCAM-negative cells. Using these nanostructured substrates, cancer cells can be reliably captured from artificial CTC blood samples, and cell viability with this platform is as high as 84%–91%. In effort to augment this performance, the SiNP substrate was combined with the herringbone structured channel (Wang et al. 2011). When driving a blood sample containing CTCs through the device, the embedded herringbone micropatterns on the channel roof induce vertical flow in the microchannel. Consequently, the contact frequency between CTCs and the SiNP substrate increases, resulting in significantly enhanced CTC capture efficiency compared to the static setting. With an optimal flow rate ($1.0 \, \mathrm{mLh}^{-1}$), a superb cell-capture efficiency ($> 95\%$) was reported using this microdevice. The optimal conditions were employed to capture and count CTCs in blood samples collected from prostate cancer patients with different degrees of tumor spread and different sensitivity to treatments. The results obtained by the devices were compared with those observed by CellSearch$^{\mathrm{TM}}$ using immunomagnetic enrichment demonstrating overall superiority over the commercial instrument. In a different approach, a polymer-based device was fabricated consisting of an array of 51 high-aspect ratio curvilinear-shaped channels (Adams et al. 2008, Dharmasiri et al. 2009, Dharmasiri et al. 2011) (**Fig. 2(d)**). CTC recognition molecules, including EpCAM antibodies (Adams et al. 2008), PSA antibodies and anti-PSMA aptamers (Dharmasiri et al. 2009) were tethered to the capture surface. The curvilinear-shaped channels improved both the cell capture efficiency and the device throughput by using parallel channels with high-aspect ratio. Using a linear velocity profile for optimal cell capture, a recovery >90% was achieved.

Drawbacks associated with affinity-based CTC isolation include limitation in capture efficiency and low throughput. However, the most severe challenge remains a clear biomarker. The most widely used CTC binding ligand, EpCAM, has been found to be expressed in ~70% tumors with different histologic type (Went et al. 2004). Similarly, cytokeratin-negative tumor cells were also found in the blood of a breast cancer patient (Fehm et al. 2002). Furthermore, CK8, CK18 and CK19 were absent in cell lines derived from disseminated tumor cells (Willipinski-Stapelfeldt et al. 2005). The absence of cytokeratins (CK) an EMT indicator, has been verified in many samples of breast cancer considered to be of a higher grade (Willipinski-Stapelfeldt et al. 2005). Thus, during EMT, the most malignant CTCs seem to cease expression of epithelial antigens; this suggests that assays designed for detecting epithelial cells in blood are prone to fail in discerning the aggressive tumor cells. Isolating CTCs requires the processing of relatively large sample volumes. Channels in microfluidic devices are typically within the range of tens to hundreds of microns. These

small sized channels are ideal for enhanced frequency of cell-surface interaction. However, the limited channel dimensions are also a bottleneck in sampling blood of large volumes ($\sim$7.5 mL). Higher flow rate facilitates improved throughput but decreases the cell-ligand interaction time. In addition, significantly increased shearing force due to higher flow rate may even prevent cell adhesion while compromising the viability of captured cells.

6.2　CTC isolation via magnetic interactions

Magnetic particles are also widely used for selective isolation of CTC. CTC isolation using magnetic interactions is different from conventional affinity-based isolation in that the homing ligands are immobilized on the surface of microbeads, rather than on a microchannel surface. In this type of assay, silica-coated magnetic particles are functionalized with highly selective ligands. CTCs are then specifically recognized by the ligands immobilized on the magnetic beads, while the CTC isolation or enrichment is accomplished by utilizing a magnetic field (Neurauter et al. 2007, Haukanes & Kvam 1993, ŠafaříK & ŠafaříKová 1999).

A method of microchip-based immunomagnetic CTC detection has been reported combining the benefits of both immunomagnetic assay and microfluidic technology (**Fig. 3(a)**) (Hoshino et al. 2011). A polydimethylsiloxane (PDMS)-based microchannel bonded to a glass coverslip was used to screen blood samples. As the blood sample flows through the microchannel closely above an array of magnets, cancer cells decorated with magnetic nanoparticles are separated from the blood flow and deposited onto the glass coverslip bottom surface, which enables direct observation of the captured cells using a fluorescence microscope. The dimensions of the microchannel combined with the sharp magnetic field gradient, in the vicinity of the magnet array with alternate polarities, lead to an effective capture of target cells. Customized Fe_3O_4 magnetic nanoparticles functionalized with EpCAM antibodies were introduced into the blood samples for binding to the target cancer cells. The blood sample was then driven through the microchip device to isolate the decorated target cells. Compared to the commercially available Veridex CellSearch^{TM} system (www.cellsearchctc.com) (which has been approved by United States Food and Drug Administration US FDA, Ref No. K031588, for monitoring the patients with metastatic breast, colorectal and prostate cancer since 2004, and has been approved by China FDA for metastatic breast cancer patients in 2012), fewer (25%) magnetic particles were required to achieve a comparable capture efficiency, while the screening speed reached 10 mL/hr. Using this method, rare cancer cells (from 1 000 cells down to 5 cells per mL) were successfully detected, and recovery rates of 90% and 86% were demonstrated for COLO205 and
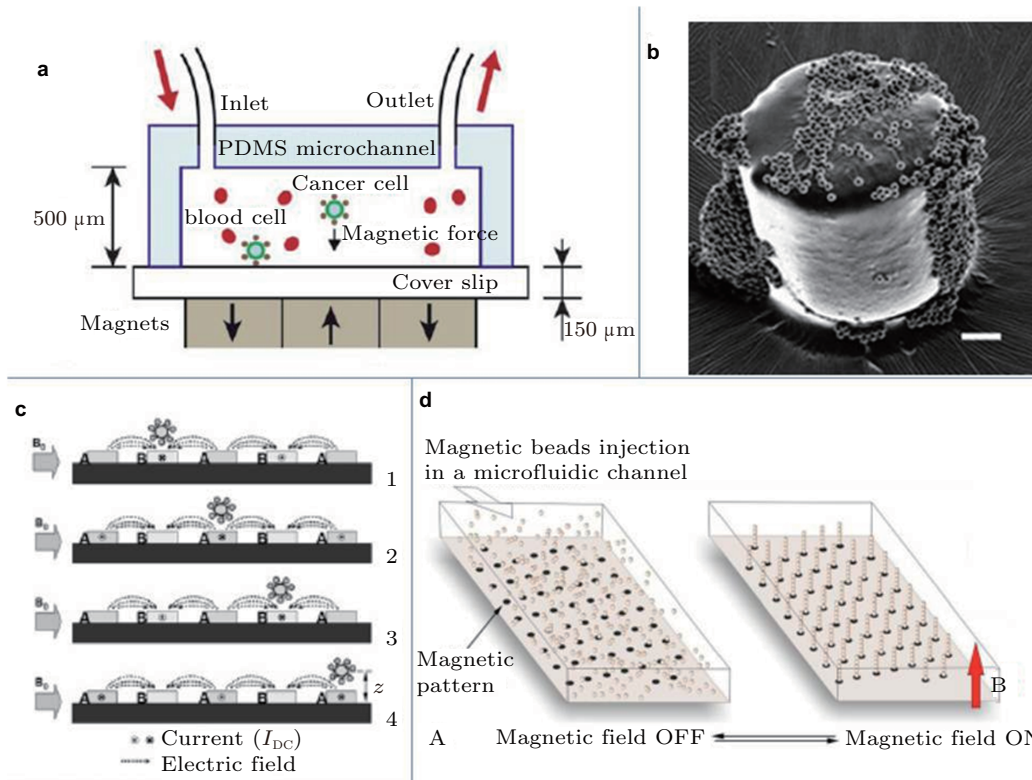
Fig. 3

Representative micro/nano devices for CTC isolation via magnetic interactions. (a) Schematic of microfluidic device isolating magnetically-labeled CTCs by magnetic field (Hoshino et al. 2011). (b) Captured magnetic beads on a ferromagnetic material encapsulated micropillar, Scale bar is 20 μm. (Xia et al. 2011). (c) Schematic of cell-particle complexes isolated and transported by a traveling magnetic field. (Stakenborg et al. 2010). (d) Principle of magnetic bead self-assembly in microfluidic channel. (Saliba et al. 2010)

SKBR3 cells, respectively. A micro magnetic activated cell sorting (microMACS) chip based on ferromagnetic material encapsulated micropillars was reported (**Fig. 3(b)**) (Xia et al. 2011). This method is capable of simultaneously producing magnetic microstructure arrays dispersed among channels that are susceptible to magnetic force, providing a uniquely selective feature. Simulations predicted that the target capture would occur precisely at the two opposing endpoints of micropillars, based on their perpendicular positioning to the flow direction and their property of maximum magnetic force. To determine the capability of the microMACS chip in capturing CTCs, SW620 human colon cancer cells were used in an *in vitro* flow model system and the capture efficiency was found to be 72.8%.

An automated modular microsystem has been developed to facilitate the detection and subsequent clinical evaluation of CTCs directly from blood (**Fig. 3(c)**) (Stakenborg et al. 2010). The proposed microsystem integrates three modules: (1) sample preparation and immunomagnetic-based cell enrichment, separation and counting; (2) reverse transcription of messenger ribonucleic acid (mRNA) to complementary deoxyribonucleic acid (cDNA) and multiplex amplification of specific cancer markers; and (3) detection of the amplified DNA. More specifically, an automated mixing chamber for immunomagnetic cell separation is introduced as a first module. The main chamber is designed for efficient mixing to enhance specific magnetic CTC isolation while retaining its viability. Following isolation of the immunomagnetic-captured CTCs, the sample volume is reduced and directly transported to a microfluidic chip via a macro- to-micro-interface. On chip, the cells are subsequently actuated and separated from unlabeled beads on the basis of differences in magnetic mobility. This process enables direct counting of the magnetically decorated cells in-flow. Following isolation and detection, the CTCs are transported to a second module for on-chip PCR. After lysis, the mRNA is transcribed to cDNA to enable simultaneous amplification of over 20 gene fragments by means of multiplex ligation-dependent probe amplification (MLPA). In the final module, the amplified DNA fragments are detected using an array of electrochemical sensors.

A unique CTC isolation method has been proposed utilizing columns of bio-functionalized super-paramagnetic beads self-assembled in a microfluidic channel onto an array of magnetic traps formed by micro-contact printing (**Fig. 3(d)**) (Saliba et al. 2010). Taking cancer cell lines as examples, a capture yield better than 94% was demonstrated together with the potential to cultivate the captured cells *in situ*. Clinical samples, including blood, pleural effusion, and fine needle aspirates were also tested using this method. The immunopheno-type and morphology of B-lymphocytes were analyzed directly in the microfluidic chamber, and compared with conventional flow cytometry and visual cytology data, in a blind test. Immunophenotyping results were fully consistent with those obtained by flow cytometry. This method provides a powerful approach to cell capture and characterization allowing fully automated, high resolution and quantitative immunophenotyping and morphological analysis. It requires at least 10 times smaller sample volume and fewer cells than cytometry, potentially increasing the range of indications and the success rate of microbiopsy-based diagnosis while reducing analysis time and cost.

6.3   CTC isolation via filtration

CTCs are different from leucocyte in cell size, density, shape and deformability. These parameters can be exploited in microsystems to isolate CTCs on the basis of mechanical restriction (Mohamed et al. 2004). In comparison with affinity-based CTC isolation, filtration-based methods use a more active fluid control strategy. In these devices, CTCs in the blood are forced to pass through the filtration mesh. These methods are usually label-free and CTC trapping, using these systems, is usually simple and cost effective. In this approach, the flow rate is typically > 1 mL/min; hence, it promises much higher throughput than that of an affinity-based assay. Furthermore, filtration-based microdevices usually require less capture area and therefore guarantee parallel sample processing in a miniaturized device.

Effective CTC isolation has been demonstrated using a microfluidic design based on the unique differences in size and deformability between cancer cells and other blood cells (**Fig. 4(a)**) (Tan et al. 2009, Tan et al. 2010). Placing physical structures along the path of blood samples in a microchannel, CTCs which are generally larger and stiffer are retained while most blood constituents are removed. The isolation efficiency is more than 80% for tests performed on breast and colon cancer cells. The operational conditions for processing blood are straightforward and permit multiplexing of the microdevices to handle several different samples in parallel. The microfluidic device is optically transparent, which makes it simple to be integrated to existing laboratory microscopes, and immunofluorescence staining can be done *in situ* to distinguish cancer cells from hematopoietic cells. Viable isolated cells are obtained on the microdevice, enabling further analysis through on-chip phenotypic and genotypic tests.

Microfilter devices for CTC detection featuring parylene membranes have also been proposed (**Fig. 4(b)**) (Zheng et al. 2011). Using a constant low-pressure delivery system, the microfilter platform was capable of cell capture from 1 mL of whole blood in less than 5 minutes, achieving 90% capture efficiency, 90% cell viability, and 200-fold sample enrichment. The captured cells retained normal morphology, as confirmed by scanning electron microscopy, and could readily be manipulated, further analyzed, or expanded on- or off-filter. CTCs were identified in 51 of 57 patients using the microdevice, compared with only 26 patients with the CellSearch[TM] method. When CTCs were detected by both methods, greater numbers were recovered by the microfilter device in all but five patients. These filter-based microdevices are both capture and analysis platforms, capable of multiplexed
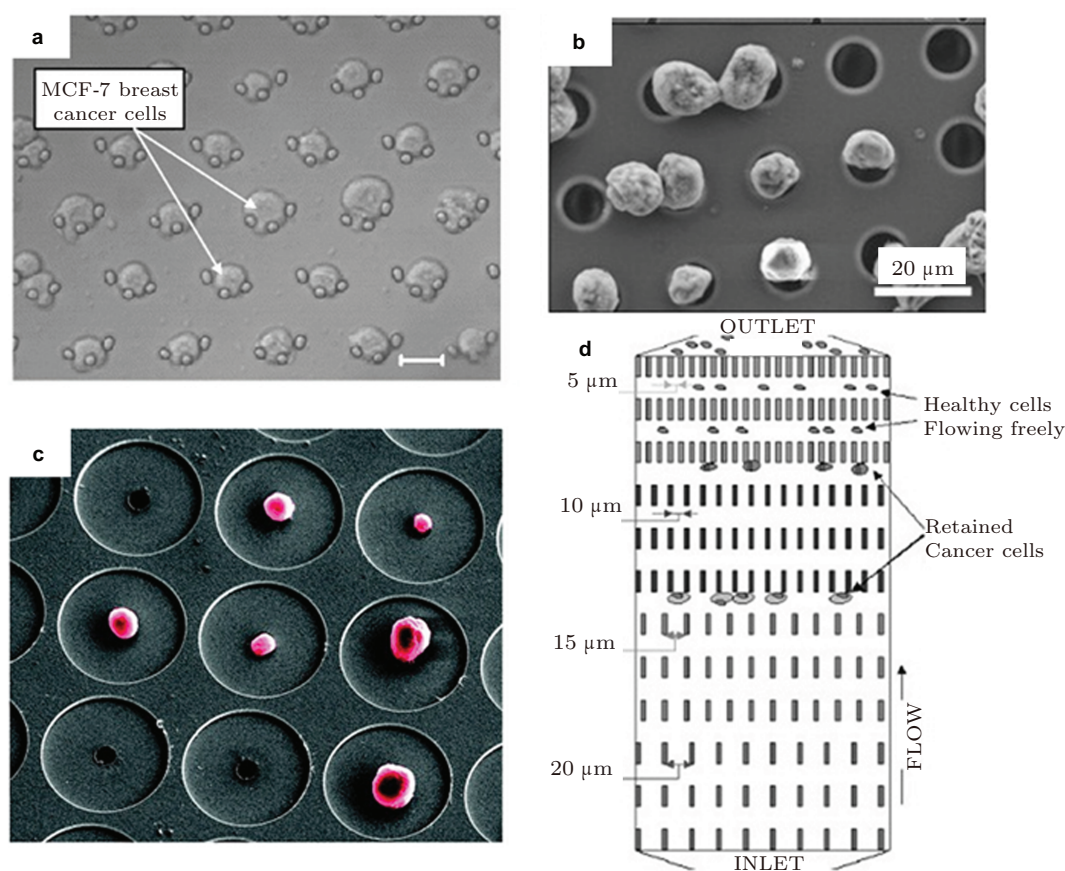
Fig. 4

Representative microfluidic devices for CTC isolation via filtration. (a) Crescent-shaped traps in a microfluidic device. Scale bar is 20 μm. (Tan et al. 2010). (b) Parylene membrane filter with LNCaP cells captured. (Zheng et al. 2011). (c) Nickel microcavity array with MCF-7 cells trapped. The microcavities are 9 μm in size, with a 60 μm pitch. (Hosokawa et al. 2010). (d) Array of microfluidic traps with varying geometrical restrictions. (Mohamed et al. 2009)

imaging and genetic analysis.

In another approach, a size-selective microcavity array for rapid and efficient detection of CTCs has been reported (**Fig. 4(c)**) (Hosokawa et al. 2010). The microfluidic device contains a nickel plate with a size-selective microcavity array, which can specifically separate tumor cells from whole blood on the basis of differences in the size and deformability between tumor and hematologic cells. The arrayed cells were then processed for image-based immunophenotypic analysis using a fluorescence microscope. This microfluidic device suc-

cessfully detected about 97% of lung carcinoma NCI-H358 cells in 1 mL whole blood spiked with 10–100 cancer cells. In addition, breast, gastric, and colon tumor cells lines that include EpCAM-negative tumor cells, which cannot be isolated by conventional immunomagnetic separation, were successfully recovered on the microcavity array with high efficiency (more than 80%). On the average, around 98% of recovered cells were viable.

A micromachined device was designed and tested to fractionate whole blood using physical attributes for enrichment and/or isolation of rare cells from peripheral circulation (**Fig. 4(d)**) (Mohamed et al. 2009). This device has arrays of four successively narrower channels, each consisting of a two-dimensional array of columns, decreasing in spacing from 20 μm to 5 μm. The first 20 μm wide segment disperses the cell suspension and creates an evenly distributed flow over the entire device, whereas the others were designed to retain increasingly smaller cells. When cancer cells were loaded into the device, all cancerous cells were isolated depending on cell size and deformation characteristics. The results demonstrated that the device is capable of retaining any cancer cell that is larger and/or less deformable than blood cells. Furthermore, intact cells can be removed from the device by reversing the flow after retaining the target cancer cells while all blood cells are flushed out of the device.

In order to achieve high-throughput CTC analysis in microfluidic systems, one important issue is the potential hydrodynamic damage of cells flowing inside microfluidic channels. Ma and the co-workers (Ma et al. 2013) has developed a high-throughput circular multi-channel microfiltration (CMCM) device integrated with a polycarbonate (PC) membrane to investigate the hydrodynamic lysing of cancer cells during the filtration. An empirical formula was proposed to predict the cell viability of the CMCM as a function of shear stress or Reynolds number. In addition, based on silicon-on-insulator (SOI) technology, a new micro-filtration chip with an array of high-density regularly spaced pores (Ma et al. 2014) was developed. Compared to the previous CMCM device with PC filter, the SOI-based system shows much better performance of CTC isolation and enumeration.

6.4   Identification and enumeration of CTCs

Since all the CTC isolation techniques cannot yield 100% specificity, it is therefore necessary to characterize the selected cells to allow reliable CTC enumeration. Methods for CTC characterization include immune-staining and quantitative reverse transcriptase–polymerase chain reaction (qRT-PCR). The criteria used in the CellSearch^{TM} as well as most microfluidic-based CTC detection assays include: round to oval morphology, a visible

nucleus (4', 6-diamidino-2-phenylindole or DAPI positive), positive staining for cytokeratins and negative staining for cluster or differentiation 45 or CD45 (Allard et al. 2004). The combination of morphology, nucleus staining and surface markers can differentiate CTCs from blood cells.

Another approach employed for the characterization of CTCs is real-time polymerase chain reaction (RT-PCR), where mRNAs are the biomarkers indicating the presence of cancer cells. The sensitivity of this approach is considered to be higher than that of immune-mediated detection and immunocytochemistry (Saliba et al. 2010). RT-PCR involves several steps: (a) CTC collection, (b) RNA extraction, (c) complementary DNA (cDNA) synthesis, (d) target sequence amplification, and (e) PCR product analysis (by gel electrophoresis or real-time amplification signal monitoring). PCR methods can identify one target cell out of 10^6–10^7 normal cells which corresponds approximately to one cell in 0.1 mL–1 mL of blood.

A qPCR-TRAP assay has been developed to amplify the telomerase activity signal from CTCs captured on micro filters (Xu et al. 2010). Using this method, the detection of telomerase activity from as few as 25 cancer cells suspended in a 7.5 mL of whole blood sample has been demonstrated. A factor which might affect telomerase activity measurement is potential cell variability in various clinical states such as inflammatory conditions or chemotherapy.

A significant disadvantage of RT-PCR is the destruction of CTCs, eliminating the possibility of counting or analyze them. Another drawback is that the selection of the marker RNAs, the transcripts indicating tumor cells in blood is not straightforward. TA good biomarker should be a transcript expressed in all target cancer cells from one particular tumor, and not expressed at all in all non-target cells, not even by illegitimate transcription (low level, non-specific transcription of certain genes) (Chelly et al. 1989). Finally, while RT-PCR has high sensitivity, it suffers from poor specificity which may result in false positives. These assays are also known to exhibit high inconsistency among different laboratories (Helo et al. 2009). Indeed, if the marker is a gene typically expressed in epithelial cells, a false-positive result will be obtained if the patient happened to have non-tumorous epithelial cells circulating in blood. Cytokeratin 19 (CK19) mRNA was detected in blood of some healthy donors, in samples of patients with haematological malignancies, and in some control subjects. Detection of Cytokeratin 19 in healthy donors has been proposed to result from the gene illegitimate transcription or enhanced secretion of cytokines that can upregulate transcription of certain genes in leukocytes (Novaes et al. 1997).

CTC enumeration without labeling has been reported using an on-chip integrated con-

ductivity sensor following release from the capture surface (Adams et al. 2008). The isolated CTCs were readily released from the antibody-functionalized surface using trypsin. The released CTCs were then enumerated on-chip using a label-free solution conductivity route capable of detecting single tumor cells traveling through the detection electrodes. The conductivity readout provided near 100% detection efficiency and high specificity for CTCs due to favorable scaling factors and the non-optimal electrical properties of potential interferences (erythrocytes or leukocytes).

6.5 *In vivo* CTCs imaging with optical MEMS technology

Noninvasive imaging of CTCs in real time as they flow through the peripheral vasculature could improve detection sensitivity by enabling analysis of significantly larger blood volumes (potentially the entire blood volume of a patient); however, to date, such analyses have proven successful only when cancer cells are labeled *ex vivo* prior to their injection (Georgakoudi et al. 2004, Novak et al. 2004). Although mitochondria-containing cells and apoptotic cells have been successfully labeled *in vivo* for detection in the vasculature (Wei et al. 2005, Zhong et al. 2005), no method has yet been developed for *in vivo* labeling and quantitation of CTCs. An intravital flow cytometry method has been reported whereby CTCs are counted noninvasively *in vivo* as they flow through the peripheral vasculature (He et al. 2007). The method involves intravenous injection of a tumor-specific fluorescently-labeled ligand followed by fluorescence imaging of superficial blood vessels to quantitate the flowing CTCs. Studies in mice with metastatic tumors suggest that CTCs can be counted weeks before metastatic disease is detected by other means. Analysis of whole blood samples from cancer patients further established that human CTCs can be selectively labeled and counted when present at ≈ 2 CTCs per mL, opening opportunities for earlier detection of metastatic disease.

Integration of optical MEMS technology is desirable for *in vivo* imaging of CTCs by miniaturized conventional microscopy. Current optical MEMS imaging technology is focused on the development of ultra-portable or needle-like MEMS-based optical micro-endoscopes for cancer or cell diagnosis *in vivo*. Two main modalities that have already been successfully realized are confocal and two-photon microscopy. Confocal microscopy is an optical imaging technique utilizing point by point illumination and a spatial pinhole to increase resolution and contrast of specimens. It offers several advantages over conventional optical microscopy, including controllable depth of field, elimination of out-of-focus image, and ability to collect serial optical sections from thick specimens (up to 150 microns) to re-construct

three-dimensional images. Two-photon microscopy allows imaging of living tissue up to one millimeter with sub-cellular to cellular resolution. Typically, a femtosecond laser is used to enable the high photon density and flux required for two photons absorption. These imaging modalities have allowed microscopes to obtain high-resolution images of living, intact tissues and have become useful for *in vivo* imaging. Miniaturization of the two modalities rely on both micro-fabricated two-dimensional MEMS scanners and miniature optics to allow intravital and clinical imaging *in vivo*. Using optical MEMS based technology, both optical imaging modalities have been miniaturized with very high acquisition frame rate (15 Hz), which could potentially be used for *in vivo* CTCs imaging (Khemthongcharoen et al. 2014). The capability of microendoscopes has been demonstrated for *in vivo* real-time vasculature imaging in mice and cancer imaging in human patients (Piyawattanametha et al. 2009, Piyawattanametha et al. 2012).

7   Summary and future perspectives

Despite the extensive advances that have been made, there are still drawbacks in microfluidic-based CTC analytical technology. First, there are limitations in isolation efficiency as each of the current capture strategies is liable to miss a portion of the CTCs. For example, EpCAM-based isolation cannot adapt for EpCAM-negative CTCs, and filtration-based methods cannot trap CTCs with small sizes. To overcome these limitations, it is tempting to combine different targeting ligands, or even different capture strategies to develop a more robust CTC isolation method.

Second, CTC characterization assays with high specificity are still highly desired. It has been pointed out that the most widely used criteria for CTC characterization, namely cells with CK(+), CD45(−) and a nucleus, cannot distinguish between circulating tumor cells and circulating epithelial non-tumor cells. Since the most malignant tumor cells most likely lose their "epithelial-specific antigens", during EMT, defining CTCs as cells expressing epithelial antigens may lead to a critical interpretation bias. Therefore, it is crucial to discover unique biomarkers for CTC characterization, which are highly specific to cancer cells.

Third, improved throughput is necessary to meet the needs for clinical applications. In pratical healthcare environment, tens or even hundreds of samples are to be processed every day; hence, an ideal CTC detection assay should feature short analysis time and parallel sample processing capacity. Lastly, the cost for CTC analysis should be largely reduced such that CTC detection can become a routine process in cancer treatment. To

date, Veridex CellSearch^{TM} is still the only FDA-approved technology for CTC detection and analysis. The CTC working group of the Cancer Steering Committee in the Biomarkers Consortium (www.biomarkersconsortium.org) has been working on development of standard language for describing CTCs and of Standard Operating Procedures (SOPs) for clinical evaluation of CTC assays prior to any testing on patient specimens (Parkinson et al. 2012). Selected microfluidic CTC systems, such as silicon CTC chip proposed by Massachusetts General Hospital/Harvard Medical School (On-Q-ity Inc., MA, USA), ClearCell^{TM} System (Clearbridge BioMedics, Singapore) have been in clinical trial. It will be expected that some low-cost microfluidic CTC systems will replace the expensive CellSearch^{TM} assay in the future.

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基于微流控技术在流体动力载荷下分离循环肿瘤细胞

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摘　要　在世界范围内, 癌症的死亡率仍在逐年上升. 循环肿瘤细胞 (circulating tumor cells, CTCs) 是指从原发肿瘤脱落并进入血液循环系统的细胞, 可能引发肿瘤转移并入侵其他正常组织和器官. 因此, CTCs 的检测结果可以作为癌症病人疗效和预后的评价指标. 但是 CTCs 的数量及其稀少, 使得 CTCs 的检测尤为困难. 在癌症转移的病人中, 每毫升血液约含有 10~100 个 CTCs. 利用经生物活性材料表面修饰的微流控器件, 可以从血液中分离出 CTCs. 这是一项跨学科的挑战, 需要来自不同学科背景的专家们共同参与, 如细胞生物学、表面化学、流体力学及微纳加工技术等. 该文首先介绍了 CTCs 的细胞生物学基础, 然后总结了当前分离 CTCs 的主要微流控技术, 包括基于细胞–配体作用、磁力作用和过滤等, 最后综述了基于微流控技术的 CTC 检测和计数、在体 CTC 成像等最新研究进展.

关键词　循环肿瘤细胞, CTC, 微流控, 癌症, 微系统, 微机电系统, 上皮间质转化, 肿瘤转移

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