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Microfluidics and Cancer: Are we there yet?

Jennifer Zhuo Zhang and Sunitha Nagrath*

Department of Chemical Engineering, University of Michigan, Ann Arbor, MI

Abstract

More than two decades ago, microfluidics began to show its impact in biological research. Since then, the field of microfluidics has evolving rapidly. Cancer is one of the leading causes of death worldwide. Microfluidics holds great promise in cancer diagnosis and also serves as an emerging tool for understanding cancer biology. Microfluidics can be valuable for cancer investigation due to its high sensitivity, high throughput, less material-consumption, low cost, and enhanced spatio-temporal control. The physical laws on microscale offer an advantage enabling the control of physics, biology, chemistry and physiology at cellular level. Furthermore, microfluidic based platforms are portable and can be easily designed for point-of-care diagnostics. Developing and applying the state of the art microfluidic technologies to address the unmet challenges in cancer can expand the horizons of not only fundamental biology but also the management of disease and patient care. Despite the various microfluidic technologies available in the field, few have been tested clinically, which can be attributed to the various challenges existing in bridging the gap between the emerging technology and real world applications. We present a review of role of microfluidics in cancer research, including the history, recent advances and future directions to explore where the field stand currently in addressing complex clinical challenges and future of it. This review identifies four critical areas in cancer research, in which microfluidics can change the current paradigm. These include cancer cell isolation, molecular diagnostics, tumor biology and high-throughput screening for therapeutics. In addition, some of our lab's current research is presented in the corresponding sections.

Background

Microfluidics handles microliter volumes in microchannels of 1 μm to 1000 μm size. In such regime, fluid flow is strictly laminar, hence concentrations of molecules can be well controlled [1]. Microfluidic technology was introduced as a biological tool in the early 1990s [2]. Since then, this interdisciplinary technology, which is well known for manipulating reagents within miniaturized platforms, has been developing rapidly [3, 4]. The material used for preparing microfluidic devices has evolved from traditional silicon and glass, to elastomers rendering the device more biocompatible and lower cost [1]. There are several inherent advantages of microfluidics, including reduced sample size and reagent consumption, short processing times, enhanced sensitivity, real-time analysis and automation [5]. One of the motivations for applying microfluidic techniques in life science is to automate the labor-intensive experimental processes similar to that accomplished in

*To whom correspondence should be addressed: Sunitha Nagrath, PhD, Department of Chemical Engineering, University of Michigan, 2300 Hayward Street, Ann Arbor, MI-48109. Phone: (734) 647-7985, FAX: (734) 764-7453, snagrath@umich.edu.

electronic circuits [2]. Polymerase chain reaction, electrophoresis on chip and DNA microarrays are among the earliest [2] microfluidic ventures. With a decade of development, microfluidic integrated systems were extended to manipulating RNA, proteins and mammalian cells using biosensors, single cell assays for disease diagnosis and prognosis, among several other applications. Biologic microfluidic devices can now be used to explore and research cancer in new and unconventional ways.

Cancer research has long been at the forefront of medical and scientific research. Its seemingly incurable nature and large prevalence in society have made cancer a popular and well-funded area of research for decades. Cancer is a chronic disease involving changes or mutations in multiple genes. It was estimated that in 2008, 12.7 million cancer cases and 7.6 million cancer-related deaths occurred globally [6]. In 2011 in the United States alone, 1.6 million people were newly diagnosed with cancers and 571,950 cancer-related deaths were projected. Prostate, breast, lung and colorectal cancers are the leading cause of cancer deaths in the US [7]. Since 2004, considerable funding has been allocated for technology advancement in search of more effective anti-cancer strategies [8]. Cancer prevention strategies, early cancer diagnosis and effective drug treatment need to be more affordable and easily accessible to improve overall survival.

Traditionally, cancer diagnosis is highly dependent upon sampling of tumor tissues or indirect quantification of proteins [9]. Often, these conventional sampling approaches are invasive which leads to tissue damage, limited access and ability to get reliable samples and cause high levels of patient discomfort. Although, proteomic and genomic research has identified a list of candidate cancer biomarkers in body fluids such as blood and saliva [10], still there is a lack of point of care devices for these assays for rapid, non-invasive diagnosis. Inherently, microfluidics is suitable for analyzing complex fluids in vitro and thus offers a non-invasive alternative for cancer diagnosis and disease management. Possible biomarkers available in the blood include DNA, miRNA, proteins and circulating tumor cells (CTCs). However, these specific cancer biomarkers are often present at low levels against massive background signals. For example, there are only 1–10 CTCs in 1ml of whole blood containing 10^6 – 10^7 blood cells [11, 12]; the ratio between targeted proteins versus the background is approximately $1:10^5$ [13]. Given this challenge of detecting the actual signal from vast noise, researchers turned to MEMS (Microelectromechanical systems) based approaches, as microfluidics is capable of performing separations with high sensitivity thus becoming a highly useful tool for this application [1]. Also because of the ease of manufacturing and low cost of microfluidic devices, biomarkers can be assessed fairly routinely with the necessary sensitivity and is evolving as one of the promising avenues to develop personalized medicine [14].

During the past decade, significant progress has been made in gaining fundamental understanding of cancer biology through advances in gene profiling [15]. To effectively target cancer and examine therapeutic response, it is vital to understand the aberrant expression profiles related to mutated genes [16]. In addition to direct DNA sequencing, mRNAs and proteins associated with specific pathways are often used to examine therapeutic response. Microfluidics offers efficient and sensitive tools to perform PCR, electrophoresis and hybridization arrays on chip to make multiplexed analyses possible [17].

In addition, Lab-on-a-Chip(LOC) technologies can enable high-throughput drug screening by spatio-temporal delivery of drugs or parallel drug stimulation with minimal cross-contamination because diffusion dominates the local solute transport [18–21]. Furthermore, LOC systems provide a powerful alternative not only to traditional cell culture, but also cell sorting and live cell arrays [22–24]. Additionally, single-cell analysis on chip can reveal cell-to-cell variability in terms of pharmacokinetic response toward different stimuli [25]. Compared to conventional cell culture techniques, microfluidics presents a better approximation to cellular environment by precisely controlling concentration gradients, extracellular matrix components and cell-cell interactions [26].

This review will outline the latest advances in microfluidics technology that have impacted cancer research and have changed the current paradigm of strategies for cancer diagnosis, monitoring and therapeutics. Particularly, approaches for isolating circulating tumor cells (CTCs), molecular diagnosis, understanding tumor biology and high-throughput multiplex screening systems will be described (Figure 1).

Four distinct areas have been defined to show the influence of microfluidics technology on cancer research. Figure 1 summarizes the four areas: one, isolation of CTCs has been approached by immunoaffinity-based, size-based and magnetic-based separation methods [27–29]; two, detection or characterization of tumor cells through molecular diagnostics can be expanded from single-cell RT-qPCR, droplet-based DNA mutation arrays to protein detection assays [30–32]; three, tumor biology can be focused on understanding tumor cell migration and cell culturing in microchannels such as multi-cellular spheroid formation [33–35]; four, high-throughput screening can be achieved including blood protein measurement, single-cell arrays for studying drug-induced apoptosis and a drug testing platform [36–38]. Next, Figure 2 demonstrates a time-evolution of microfluidic technologies for cancer across the past 20 years. Recently many technologies have been emerging to better serve cancer diagnosis and treatment [20, 27, 36, 38–56]. Below, specific microfluidic approaches for each area will be discussed beginning with circulating tumor cell isolation.

Microfluidic technologies for Isolation Circulating Tumor Cells (CTCs)

Recently, several hypotheses have been developed regarding cancer metastasis and progression. CTCs, tumor cells shed by primary tumors into circulation, have been identified as the lethal drivers in the metastatic cascade. They can ultimately lodge, invade and proliferate in distant secondary sites initiating metastatic lesions. Metastasis is the leading cause of cancer related deaths [57]. In some cancers, metastasis may occur in the early stage of tumor progression[58]. In addition, metastatic cells acquire mutations beyond those initiated within primary tumors[59]. Therefore detecting CTCs can be extremely valuable to cancer diagnosis in early stages and help with treatment decisions. The emerging microfluidic technologies can isolate CTCs, based on their biochemical or physical properties, using a variety of methods.

Immunoaffinity-based Isolation

One of the major CTC isolation method is based on antibody-antigen interactions. The most commonly used surface antigen is Epithelial Cell Adhesion Molecule or EpCAM, first

identified in the late 70's [60]. EpCAM is overexpressed in breast, colon, lung, prostate, gastric, ovarian and renal carcinomas [60–62] and hence widely employed as the target antibody in almost all immunoaffinity based CTC isolation strategies.

Early in 2004 one of the first CTC detection technologies, CellSearch, demonstrated CTC-based diagnostic potential by separating CTCs using EpCAM-coated magnetic beads and correlating the number of isolated CTCs to prognosis in breast cancer patients. It is the only device approved by the U.S. Food and Drug Administration for isolation of CTCs in metastatic breast, colon and prostate cancers [63]. In 2007, a microfluidic-based CTC capture device, which consisted of 78,000 EpCAM-coated microposts embedded on a silicon chip, was first published in *Nature* [27]. The chip can capture cancer cells from milliliters of unprocessed whole blood with high sensitivity and purity. The captured cancer cells are maintained in an appropriate condition for molecular analysis through immunostaining, or DNA/RNA extraction. The chip successfully detected CTC in all but one of 116 blood samples from 68 patients with metastatic lung, prostate, pancreatic, breast and colon cancer.

Later in 2008, it was shown that the CTC-chip technology was successfully applied to monitor the epidermal growth factor receptor gene (EGFR) mutations in patients with non-small-cell lung cancer. Sufficient DNA was isolated from the captured CTCs to allow allele specific assay testing and in few instances direct sequencing. Not only were rare somatic genetic mutations detected in 19 out of 20 EGFR positive patient samples but the device also detected secondary resistant mutation from 11 out of 12 patients, who developed resistance to tyrosine kinase inhibitors [64]. This type of information that is vital for diagnosis, prognosis, and therapeutics was earlier provided only by invasive tissue biopsies. Non-invasive genotyping or so called “blood biopsies” in patients was made possible with the CTC-chip.

In 2010, another step forward was taken in the clinical development of the CTC-chip by developing an automated strategy to characterize CTCs of prostate cancer [65]. CTCs were fixed and stained with the prostate-specific antigen (PSA) and DNA content. The chip was imaged in a semiautomated fashion and CTCs were characterized by an image processing algorithm in terms of fluorescence intensity, cell shape and other morphological traits. Applying this method enabled easier monitoring of CTC levels for different patients during the course of therapy.

The CTC-chips discussed so far immobilized antibodies on microposts through surface chemistry. Antibodies linked to magnetic arrays can self-assemble in plain microchannels [66]. This system can sort B-lymphocytes from patient with leukemia and lymphoma. Also cancer cells were separated from a mixture of cancer and endothelial cells with an efficiency of 80% using the same device. Another immobilization technique was demonstrated by Dharmasiri et al. using aptamers to target membrane proteins expressed on prostate cancer cells and captured cancer cells with high efficiency and purity [67]. Dickson et al. reported a streptavidin coated microfluidic device can isolate cancer cells from blood cells incubated with biotin-tagged anti-EpCAM [68].

Despite the ability to specifically target CTCs, another challenge for CTC isolation remains. CTCs are very rare (1 to 10 per mL of whole blood) compared with billions of white blood cells and red blood cells. The rarity poses a significant engineering problem in designing a device to capture CTCs with high specificity and purity and simultaneously keep the cells viable for subsequent molecular analysis [11]. Researchers have been developing sophisticated microfluidic devices to address these issues.

Stott *et al* made a high-throughput polydimethylsiloxane (PDMS) based CTC-chip with enhanced capture efficiency and optical properties [69]. The microchannel was fabricated into a herringbone shape which generated passive mixing via microvortices. It can process larger volume of blood as compared to the micropost-based CTC-chip while maintaining the same capture efficiency. For example, the herringbone chip can maintain >40% capture efficiency at flow rate up to 4.8mL/hr but efficiency of CTC-chip dropped significantly above 2–3mL/hr. After being captured, cancer cells were viable and intact for molecular characterization and imaging.

Gleghorn *et al* reported a geometrically enhanced differential immunocapture (GEDI) chip to capture prostate CTCs with high-efficiency and high-purity [70]. The researchers optimized the displacement, size and shape of posts to maximize the interactions between large CTCs (15–25µm) and the antibody coated surface while small blood cells (4–18µm) can escape capture. Testing clinical blood samples exhibited increased capture efficiency and purity on the GEDI device.

Myung *et al* demonstrated immobilization of E-selectin and anti-EpCAM on the microfluidic channels enhanced capture of CTCs. E-selectin induced rolling of leukocytes and cancer cells at different velocities resulting in increased antibody accessibility to cancer cells [71]. The cancer cells tended to roll faster as the shear stress increased while the rolling velocity of leukocyte remained stable. Therefore, this approach achieved separation of leukocytes and CTCs with increased cell capture efficiency.

Dharmasiri *et al* have recently reported an integrated microfluidic system which incorporates immunoaffinity-based capture, enzymatic release, conductivity enumeration and electrokinetic enrichment of colorectal CTCs [54]. This method allows consequent manipulation and molecular profiling of CTCs using PCR coupled with a ligase detection reaction (LDR) assay. Since this system contains an electrokinetic enrichment component, it can concentrate the mass-limited DNA samples extracted from CTCs. They were able to detect the KRAS oncogene mutation in the SW620 cell line but not in the HT29 cell line, which was consistent with the known genotypes.

Hoshino *et al* utilized immunomagnetic separation mechanism to isolate cancer cells in a microchip. Blood samples containing spiked cancer cells were incubated with magnetic nanoparticles conjugated to anti-EpCAM prior passing through the device. This device can isolate as few as 5 cells per mL of blood and can be operated at 10mL/hr flow rate without a significant reduction in capture rate [72].

Most recently, Kang *et al* presented a novel CTC isolation approach which incorporates magnetic separation with microfluidic devices that permits removal of captured CTC and

culturing in vitro [73]. The device consisted of a main channel flanked by two rows of dead-end side chambers for magnetically labeled CTC collection. The mouse blood sample was first treated with EpCAM coated magnetic beads followed by flow through the isolation channel. After that, CTCs can be released by moving the magnet to the opposite side of the device. CTCs were then cultured and checked for viability.

In our lab, poly-dimethylsiloxane (PDMS) is used to construct microfluidic devices that can capture low numbers of cancer cells from whole blood using the EpCAM based immunoaffinity capturing principle. For example, a non-small cell lung cancer (NSCLC) cell line, H1650 cells were spiked into whole blood and run through a microdevice containing thousands of EpCAM coated microposts. The capture efficiency was around 97%. The chip was then immunostained with cytokeratin 7/8, CD45 and corresponding secondary antibodies followed by 4',6-Diamidino-2-Phenylindole, Dilactate (DAPI) for nuclear counterstaining (Figure 3). Compared with the previous reported results [27], this PDMS based CTC capture chip exhibited higher capture efficiency and purity, more than 90% as compared to previous 60–70%. Also this device can be easily fabricated with low cost. And because of the transparent materials used, the quality of imaging is improved.

The major drawback of immunoaffinity-based isolation is that the expression level of EpCAM on CTCs varies among cancer types and for a given cancer. Some of the CTCs might not express EpCAM, particularly cells that undergo epithelial-mesenchymal transition (EMT) [74]. Therefore, immunoaffinity CTC-chips might miss some subpopulations of CTCs which may carry important genetic information about primary tumors. Utilizing size-based separation and filtration in addition to immunoaffinity methods may increase capture of CTCs with minimal loss of low EpCAM expressing cells.

Size-based Separation

CTCs are often larger in size and may have a different specific gravity than blood cells therefore they can be separated from blood cells either by physical filtration or by hydrodynamic forces [75]. One advantage of the size-based separation is that cells can be enriched without using a specific biomarker. With hydrodynamic separation, there is an added advantage that the system can be operated at relatively high flow rate which is valuable to enrich rare CTCs. Furthermore, isolated CTCs can be collected without compromising cell viability or gene expression profile thereby enabling off-chip cellular and molecular characterizations. Microfluidic devices enable sorting of CTCs based on size followed by single cell analysis or cell culture on chip as well.

Zheng et al presented an efficient membrane microfilter device made of parylene-C for isolation of prostate cancer cells from whole blood [76]. The membrane filter contains 16,000 evenly distributed pores of 10µm diameter and 20µm space in between. The membrane was integrated with electrodes for direct electrolysis of the retained cancer cells and then polymerase chain reaction (PCR) was carried out on the cell lysate. In successive approaches, researchers used two-layer membranes to filter viable prostate and breast cancer cells [77]. The captured cells were cultured on device for 2 weeks. Two issues arose with increasing volumes of blood processed; the membrane was easily clogged and whole blood needed to be diluted before filtering.

Kuo et al demonstrated a microfluidic filtration system which can separate breast cancer cell spiked into whole blood with 50–90% recovery rate [78]. The device consisted of a serpentine channel interconnected with two outer filtrate channels with rectangular apertures. The force experienced by cells during the filtration process was carefully assessed and the dimensions of the apertures were adjusted accordingly to minimize cell damage.

Hur et al presented a device to enrich cancer cells in diluted blood by a factor of 5.4 [29]. They utilized the principle that distinct focusing positions of deformable particles can be created by a balance between inertial lift forces and viscoelastic forces in microchannels with high aspect ratio. Despite high throughput and ease of operation, the blood samples needed to be diluted to avoid defocusing caused by cancer and blood cell interactions.

Lim et al utilized a particle tracking analysis (PTA) method to study the particle focusing in microchannels [79]. Polystyrene beads, white blood cells and prostate cancer cells (PC-3) were tested in both diluted and whole blood generating two-dimensional focusing profiles as guidelines for isolating cells from whole blood.

Despite some key advantages of size-based separation, the performance of this technique is still limited due to the heterogeneity of CTCs in size and morphology [80]. To overcome this challenge, additional downstream processes might be needed to increase detection accuracy and sensitivity.

Other MEMS based Methods

Talasaz et al demonstrated a magnetic sweeper utilizing an immunomagnetic separation mechanism with enhanced purity and recovery rate [81]. The device was composed of magnetic rods sweeping wells of a six-well plate to capture magnetically labeled breast cancer cells from blood. The cells were released and underwent genomic sequencing and other molecular analyses. Moon et al. combined both hydrodynamic focusing and dielectrophoresis to isolate high purity cancer cells from blood at high flow rates [82]. Diluted blood was passed through a multi-orifice microchannel for separating blood cells from cancer cells by different equilibrium positions of cells. Cancer cells, now pooled with fewer blood cells were then flowed into a non-uniform electric field for further separation. The combined modules achieved efficient enrichment of cancer cells in a reduced time period. Chen et al. presented a microfluidic disk to negatively deplete non-tumor cells via immunomagnetic principles to achieve isolation of rare cancer cells [83]. Non-target cells were labeled with magnetic beads and as samples passed through a multistage magnetic field, those cells got trapped. Compared to positive immunoaffinity selection, negative depletion accommodates the need to capture CTCs that don't express the typical surface markers, such as cells undergoing epithelial-mesenchymal transition (EMT) [84].

Microfluidic Approaches for Molecular Diagnosis of Cancer

In addition to cellular approaches, other biomolecules are monitored for cancer diagnostics such as circulating tumor DNA, microRNAs, proteins and serum microvesicles [85–88]. Microfluidics exhibits high sensitivity and accuracy for detecting cancer specific biomarkers present at low concentrations. Additionally, microfluidics can be developed into point-of-

care devices with reduced cost which will likely lead to routine minimally invasive-clinical testing.

Yung *et al.* demonstrated a microfluidic digital PCR platform for detecting rare EGFR mutations from tumor tissue and plasma in non-small cell lung cancer patients [89]. White *et al.* presented an integrated microfluidic device to perform RT-qPCR with high throughput and precision [30]. The device consisted of 300 parallel assay chambers processing 20 μ l samples. It was used to detect single-cell mutations from metastatic breast cancer cells. Pekin *et al.* recently showed a droplet-based microfluidic device to quantitatively and sensitively detect rare KRAS mutations of tumor DNA encapsulated in droplets [31]. The mutant DNA and wild-type DNA are readily identified by reading the fluorescence from the array of droplets. This technology reduced the cost and simplified the procedure of the digital PCR process.

MicroRNAs (miRNAs) are small, non-coding RNA molecules that regulate gene expression and can play an important role in cancer development [90]. Circulating miRNAs in blood are stable and can be regarded as a potential biomarker for early cancer detection [91]. Schrauder *et al.* utilized a commercial microfluidic-based array, Geniom Biochip, to perform miRNA-profiling from the blood of 48 patients with early stage breast cancers [92]. They were able to detect 46 down-regulated and 13 up-regulated miRNAs in the patients compared to healthy controls. They found miR-202 was significantly up-regulated and may be involved in carcinogenesis.

In another study, Mitchell *et al.* extracted miRNAs from the blood serum of prostate cancer patients followed by quantitative RT-qPCR measurement using TaqMan microfluidic arrays [86]. They found that miR-141 is expressed specifically in prostate tumors and can serve as a cancer-specific biomarker. Despite the many advantages miRNA detection may offer, challenges are that the sensitivity, specificity and accuracy of the system is still limited for processing the small amount of miRNAs extracted from whole blood.

Digital microfluidic devices, which incorporate electrodes and use electrical forces to move liquids, can be applied to sensitively detect rare amount of hormones for cancer diagnosis. For example, the level of estrogen is an important risk indicator of breast cancer. Mousa *et al.* demonstrated a highly sensitive droplet-based estrogen detection assay to process tissue samples, blood, and serum within a short period of time [32]. The device used electrical forces to drive sample manipulation exploiting the conductive nature of most biologically relevant liquids.

The search for blood biomarkers for cancer diagnosis has been extended to microvesicles that are derived from cells and circulate in blood. Microvesicles (exosomes) are small membrane-bound particles that are abundant in plasma. The size of exosomes is 60–100nm [93]. miRNAs, proteins and lipids are packaged in exosomes making them potential biomarkers for cancer detection [94]. Chen *et al.* demonstrated a microfluidic device that can isolate microvesicles (exosomes) from blood serum of glioblastoma multiforme (GBM) patients via anti-CD63 functionalized surface. RNA was extracted from the captured exosomes followed by RT-PCR analysis [95]. This microfluidic isolation approach provided

quick exosome identification and enabled the extraction of high quality RNA. One major challenge of isolating exosomes is the ability to discriminate exosomes specifically from other similarly sized biological structures.

Overall, microfluidic devices are able to detect small molecules present in complex bodily fluids with accuracy and specificity. This alternative approach may lead to advances in monitoring cancer progression, personalized treatments based on tumor make up, and insight into the how cancer genetics alter through therapeutics.

Microfluidic Systems to Explore and Understand Cancer Biology

Microfluidics can mimic the physiological cues in the cellular environment through spatial and temporal control over gradients of soluble factors and cell-cell contacts in extracellular matrix [26]. Additionally, components for downstream analysis such as imaging or molecular characterization can be connected with cell culture module to make an integrated system. All of these factors make microfluidics an excellent tool for studying cell biology. When it comes to exploring cancer cell biology, various models have been developed to investigate cancer cell migration, angiogenesis and tumor microenvironment.

Chaw et al performed a quantitative study on tumor cell migration through microgaps with or without Matrigel-endothelial cells lining [50, 96]. Cancer cells were cultured in side channels with serum deficient medium and individual cells migrated towards the central channel which contained complete medium. They were able to calculate the cell migration rate and observe cell deformation which provided novel drug targets for metastasis. Another cell migration study in microfluidic channels was presented by Irima et al in which individual cancer cells were mechanically confined within channels then, within several hours, cell movement was observed as fast and persistently in one direction [35]. This device can be viewed as a cancer-cell invasion assay mimicking cancer cell migration from primary tumor sites at the onset of metastasis. Most recently, Huang et al reported a compartmentalized microfluidic device to study brain tumor stem cell migration and to isolate the stem-like cancer cells for subsequent culturing and analysis [97].

To gain insight into the effects of tumor microenvironment on cancer progression, a microfluidic system that captured the transient interactions between endothelial cells and cancer cells during metastasis was developed [98]. The microfluidic device consisted of two PDMS layers sandwiching a layer of porous polyester membrane. In particular, the effects of chemokine CXCL12 acting on endothelium through CXCR4 receptors was investigated and shown to regulate the organ specific homing of metastatic cancer cells. Using the two-layer design principle, the same group reported the formation of co-cultured 3D spheroids of prostate cancer cells, osteoblasts and endothelial cells in microfluidic channels as a model resembling the metastatic prostate cancer bone microenvironment [33]. The researchers were also able to identify the cancer stem cell subpopulations inside the spheroids. Compared with 2D co-culture environment, the 3D spheroids can better mimic the physiologic microenvironment in terms of cell proliferation rate, intercellular interactions and preserving stem-cell like subpopulations.

Huang et al presented another microfluidic design to co-culture cells in 3D [99]. Different cell types were loaded in distinct parallel channels connected by a series of juxtaposed channels partitioned by regularly spaced posts acting as a partition to the cell loading channels. It was observed that macrophages invaded toward the breast cancer cells after one week of co-culturing. The purpose of this co-culture device is to mimic the interactions between tumor cells and their surrounding stromal environment, and by varying environmental cues, epigenetic changes can be revealed.

Chung et al demonstrated another cellular environment induced migration assay using 3D collagen gel separated flow channels [34]. They were able to culture endothelial cells in the middle channel and stimulate their migration through the gel region by either a gradient of growth factor or by culturing cancer cells (MTLn3 or U87MG) or muscle cells in the side channels. Quantitative assessment of the effect of applied growth factor gradients, the stiffness of the gel and the co-culture environment was performed.

Sung et al constructed a microfluidic co-culture system that enabled patterning of cancer cells (MCF-DCIS) and fibroblasts in adjacent laminar flow regimes [100]. It was shown that fibroblasts promoted invasive transition of the cancer cells. Domenech et al demonstrated the ability to use a microfluidic co-culture system to understand the paracrine signaling between cancer cells and stromal cells [56, 101]. In particular, the hedgehog signaling between prostate cancer cells and myofibroblasts was captured for the first time in vitro.

One of the challenges of culturing cells in microfluidics comes from controlling the tiny environment surrounding the cells. Parameters like medium composition, shear stress, chemical gradients and temperature are of important consideration when designing the system [102, 103]. However, once the appropriate conditions are obtained microfluidic devices provide a tailored, controlled environment for cellular studies.

Furthermore, microfluidics provides an excellent opportunity to easily pattern cells to create the desired environmental cues for cell growth and proliferation. In our lab, we used laminar flow [100, 104] to pattern multiple cell types in microchannels and were able to study the cellular interactions between cancer cells and non-tumorous cells existing in the tumor microenvironment. For example, MCF7 breast cancer cells were co-cultured with fibroblasts in a side-by-side pattern in a 3D gel environment. After 5 days of culturing, the cells reached confluence. MCF7 cells were immunostained with cytokeratin 7/8, EpCAM and DAPI. Fibroblasts were only stained with DAPI (Figure 4).

High-throughput Biomarker and Drug Screening

High throughput multiplex screening using microfluidics has gathered momentum recently as a method in cancer research. The microfluidic high-throughput screening system requires fewer reagents, small sample volumes and can process multiple compounds with various concentrations in shorter time. Also the system can be automated to increase the efficiency of anti-cancer drug development. Cell-based microarrays enable large-scale single-cell study to identify rare cancer subpopulations, like stem cells and progenitor cells [26]. Single cell analysis has gained increasing attention for elucidating the heterogeneity among cancer cells.

Fan et al developed a point-of-care diagnostic device which detects multiple proteins from small blood serum samples within 10 minutes [36]. A DNA-encoded antibody library technique was used to construct the barcode arrays for immuno-detection of 12 tumor-associated biomarkers. 22 patients with breast and prostate cancers were examined and confirmed the reliability of the system. Jokerst et al presented another approach for multiplex serum protein detection employing a quantum dot-related biosensor [105]. Stern et al proposed a label-free biomarker detection platform which was composed of a microfluidic purification chip for pre-concentration and a nanosensor chip for detection [106]. This system can handle a 10 μ l of blood sample for quantitatively detecting multiple soluble proteins present in blood in 20 minutes. Two model biomarkers PSA and CA15.3 were tested in the device and exhibited highly sensitive and simultaneous detection. Wlodkowic et al demonstrated a microfluidic single-cell assay to study tumor cell apoptosis and screen for anti-cancer drugs [38]. The device consisted of an array of 440 traps that can immobilize leukemia cancer cells for consequent characterizations. This platform enabled real-time imaging and monitoring of apoptosis.

Kim et al developed an automated microfluidic cell culture array consisting of 64 chambers which can examine 64 pair-wise drug combinations from two input streams [37]. Prostate cancer cells PC3, were tested with two sensitizer drugs and a cell-death inducing drug, TRAIL. The two-layer PDMS device was controlled by LabVIEW software enabling large-scale testing of different combinations of drugs which can be valuable for identifying effective therapy. Miller et al approached high-throughput drug screening using a droplet-based microfluidic platform [107]. The system made use of the Taylor-Aris dispersion mechanism to generate different concentration of drugs encapsulated in droplets. The concentration of drugs can be indicated by the fluorescence from enzymatic reactions. Higher fluorescence corresponds to a better inhibition effect by the drugs. Potential inhibitors towards protein tyrosine phosphatase 1B, a known diabetes and cancer target, were tested and the dose-dependent response was plotted.

Overall, microfluidics based high throughput platforms are very promising and highly attractive for rapid testing of drugs and biomarker discovery. This is one of the rapidly evolving filed along with cell based diagnostics.

Challenges and Future Directions

Despite the promise of emerging technologies for cancer diagnosis and therapy, most of them haven't been validated with relevant clinical samples and almost none reached clinical trials [108]. There exists a considerable gap between laboratory investigation and clinical application. One of the key challenges is that nearly all samples of clinical relevance are complex in nature and handling of these in microfluidic environment needs special design consideration. It is of great importance to take into account the complexities involving patient samples from the conceptual stage itself. In a sense, a bridge needs to be built to cover the gap during technology development. This will make clinical implementation of the technology much more feasible. Access to clinical samples while testing and optimizing devices is very important as it can reduce development time, by avoiding total re-

optimization and re-designing, if the required tools, assays and parameter space are realized in the initial optimization stage itself.

Cancer diagnosis can be viewed as the first stage of treatment and is important for designing personalized medicine. The ability to detect cancers at early stage, to target validated cancer biomarkers, and to characterize certain mutations leading to drug resistance in tumor progression has always been a great effort in cancer research and will remain a challenge for the future. Low-cost and point-of-care diagnosis tools and technologies will be desperately needed to enable cancer management and reduction in mortality rates.

Microfluidics holds great promise for miniaturization and automation through handling small amount of materials and incorporating control systems. Microfluidics can detect CTCs from peripheral blood and collect genetic information to validate stage-specific markers for diagnosis and monitoring treatment response or relapse. The next generation of microfluidic devices would possibly make use of multiple biochemical and biophysical cues that are unique to cancer biomarkers to achieve high detection efficiency, high cell viability, and high throughput, which would enhance the clinical relevance of microfluidic technologies for cancer detection. A high throughput technique for immunoaffinity based assay should be able to process approximately at 10mL/hr such that meaningful volumes can be processed in a reasonable time. The assay can take anywhere up to 3hrs for completion from the sample input to enumeration. With an emerging interest to apply microfluidic-based technologies in cell biology, the devices can be made effective tools for understanding basic tumor biology in terms of tumor microenvironment, gene redundancy, and tumor stem cells. All of these would foster drug and therapeutic development in the battle with cancer.

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Figure 1.

Role of microfluidic technologies in cancer research. Isolation of CTCs using immunoaffinity-based[27], immunomagnetic-based [28] and size-based [29] methods. Molecular Diagnosis: on-chip single-cell RT-qPCR carried out in each of the reaction chambers [30], droplet-based PCR for detecting rare mutations [31], droplet-scale estrogen assay for measuring small amounts of tissue [32]. Tumor Biology: formation of 3D co-culture spheroids for studying the metastatic microenvironment of prostate cancer [33], cell migration platform to study the effect of co-culture environments [34], cancer cell migration in microcapillary array in conditions of mechanical confinement [35]. High-throughput Screening: integrated blood barcode chip to detect plasma proteins [36], programmable cell culture array for drug screening [37], single-cell array composed of micromechanical traps to screen anti-cancer drugs that induce apoptosis [38].

Figure 2.

Timeline of development of microfluidics based technologies for cancer [20, 27, 36, 38–56].

Figure 3. Capture efficiency and purity of lung cancer cells H1650 spiked into whole blood (left), Immunofluorescence images of H1650 cells with blood cells (right) (green: cytokeratin 7/8, red: CD45, blue: DAPI).

Figure 4. Microfluidics based co-culture systems. MCF7 cells in the middle co-cultured with fibroblasts on the sides patterned by laminar flow (green: EpCAM, red: cytokeratin 7/8, blue: DAPI). MCF7 cells are indicated by red cytokeratin, green EpCAM and blue DAPI nucleus. Fibroblasts are only stained with DAPI surrounding the cancer cells on the two sides. Cancer cells tend to grow into the fibroblasts lanes.

Table 1

Microfluidic technologies for isolation of CTCs

List of Technology	Principle	Application	Clinical Study
CTC-chip [27]	Immunoaffinity	Isolation of CTCs (1ml/hr flow rate, 60–65% efficiency, 50% purity from patient samples)	68 patients with metastatic lung, prostate, pancreatic, breast and colon cancer
CTC-chip [64]	Immunoaffinity	Identify EGFR mutations	27 patients with metastatic non-small-cell lung cancer
CTC-chip [65]	Immunoaffinity	Automated imaging of captured CTCs	62 patients with prostate cancer
CEE microchannel [68]	Immunoaffinity	Isolate of cancer cells from blood cells	N/A
Herringbone-chip [69]	Immunoaffinity	High-throughput mixing and isolation of CTCs (1.5–2.5ml/hr flow rate, 90% efficiency, 14% purity from spiking cells in blood)	15 patients with metastatic prostate cancer
Self-assembled magnetic arrays [66]	Immunomagnetic	Isolation of B-lymphocytes (9ul/hr flow rate, 94% yield)	7 patients with B-cell hematological malignant tumors (leukemia and lymphoma)
Aptamer selection chip [67]	Immunoaffinity through aptamers	Isolation of prostate cancer cells from blood (2ml/hr flow rate, 90% recovery, 100% purity—cell line test)	N/A
Geometrically enhanced differential immunocapture (GEDI) chip [70]	Immunoaffinity	Isolation of prostate cancer circulating tumor cells (1ml/hr flow rate, 85% efficiency, 68% purity from spiking cells in blood)	Blood samples of castrate-resistant prostate cancer patients
E-selectin biomimetic chip [71]	Immunoaffinity & Biomimic	Isolation of cancer cells from mixture of leukocytes (1.2ml/hr flow rate, 35% efficiency)	N/A
Integrated CTC selection chip [54]	Immunoaffinity & electrokinetics	Isolation, enumeration, enrichment of CTCs (1.5ml/hr flow rate, 96% efficiency) PCR/LDR detection of KRAS colorectal cancer cell mutations	N/A
Immunomagnetic chip [72]	Immunomagnetic nanoparticles	Capture cancer cells spiked in blood	N/A
Micromagnetic chip [73]	Immunomagnetic	Isolation CTCs and release for culturing (90% efficiency)	N/A
Membrane microfilter [76, 77]	Size	Separation of cancer cells from blood (89% recovery)	N/A
Filtration chip [78]	Size & Deformability	Separation cancer cells from blood cells (0.72–0.96ml/hr flow rate 50–90% recovery.)	N/A
Deformability-based chip [29]	Size & Deformability	High-throughput separation and enrichment of CTCs from diluted blood (1.5–27ml/hr flow rate, 96% yield, 3.2–5.4 fold enrichment)	N/A
Particle focusing chip [79]	Size	Use particle trajectory analysis to study cancer cell focusing in whole blood	N/A
MagSweeper [81]	Immunomagnetic	Isolation and enrichment of breast cancer cells from whole blood (process 9ml blood per hour, 62% efficiency, 51% purity)	Blood samples from 17 female patients with metastatic breast cancers

List of Technology	Principle	Application	Clinical Study
MOFF and DEP chip [82]	Size and Dielectrophoretic properties	Isolation of breast cancer cells from blood (126ul/min flow rate, 99% efficiency)	N/A
Negative selection disk [83]	Immunomagnetic	Isolation of breast cancer cells from mononuclear cells mixture (60% yield)	N/A

Table 2

Emerging microfluidics based approaches for molecular diagnosis of cancers

List of Technology	Principle	Application	Clinical Study
Microfluidic digital PCR [89]	BioMark System (Fluidigm)	Detect EGFR mutations	Plasma and Tissue samples from patients with non-small cell lung cancer
Microfluidic single-cell RT-PCR [30]	300 parallel chambers	Single nucleotide variant in metastatic breast cancer cells	N/A
Droplet-based quantitative PCR microfluidics [31]	Compartmentalization of DNA in droplets	KRAS mutations in six cancer cell lines	N/A
Microarray-based miRNA profiling [92]	Geniom Biochip	Identify miRNA in whole blood	48 early stage breast cancer patients
Microfluidic screening of miRNAs [86]	Microfluidic TaqMan miRNA qRT-PCR array	Detect tumor-derived miRNAs from plasma and serum	25 patients with metastatic prostate cancer
Digital microfluidic device for estrogen detection [32]	Droplet-based electrokinetic assay	Detect estrogen level in tissue samples, blood, and serum	Breast tissue samples from 2 breast cancer patients
Microvesicle-isolation chip [95]	Immunoaffinity	Isolation of serum microvesicles and RT-PCR analysis of point mutations	Brain tumor specimens from patients with glioblastoma multiforme

Table 3

Microfluidic platforms to explore the fundamental biology of cancer

List of Technology	Application	Clinical Significance
Microfluidics for tumor cell migration [50, 96]	Studying of tumor cell migration and deformation through microgaps	Cell membrane as a novel drug targeting
Cancer cell migration assay [35]	Cancer cell migration in one direction within confined microchannels, quantify the cancer cell motility	Drug targets for cancer invasion, motility screening
Compartmentalizing cell migration microfluidics [97]	Brain cancer stem cell migration and morphology characterization	Study brain cancer stem cell infiltration of brain parenchyma
Microfluidic vasculature system [98]	Mimicking circulating cancer cell targeting on endothelium	Drug targeting, organ specific targeting
Microfluidic co-culture [33]	Formation of 3D spheroids of prostate cancer cells, osteoblasts and endothelial cells	Drug testing platform
Microfluidic co-culture [99]	3D culture of metastatic breast cancer cells with macrophages in patterned hydrogels	Drug testing and targeting
Microfluidic co-culture and angiogenesis [34]	Study endothelial cell migration with cancer cell co-culture	Understand capillary morphogenesis
Microfluidic co-culture [100]	Understand fibroblasts associated cancer cell progression	Test for cancer progression inhibitors
Microfluidic co-culture [56, 101]	No-flow microfluidic cell culture system enabling hedgehog signaling between prostate cancer cells and fibroblasts	Study the paracrine signaling pathways between tumor and stromal cells

Table 4

High throughput MEMS based strategies for biomarker discovery and drug screening

List of Technology	Principle	Application	Clinical Study
Integrated barcode chip [36]	DNA-encoded antibody library technique	Detect multiplex tumor- associated proteins from blood serum in short time	22 patients with breast and prostate cancer
Nano-Bio-Chip [105]	Quantum dot immunoassay	Detect colon, breast and ovarian cancer biomarkers	N/A
Label-free detection chip [106]	Immunoassay	Detect PSA and CA15.3 markers for prostate and breast cancer	N/A
Single-cell microassay [38]	Mechanically trapping	Real-time monitoring apoptosis of cancer cells	N/A
Cell culture microarray [37]	Concentration gradients	Drug testing	N/A
Droplet-based drug screening microfluidics [107]	Taylor-Aris dispersion, droplet generation	Screen a library of potential inhibitor towards protein tyrosine phosphatase 1B	N/A