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Tumors on chips: oncology meets microfluidics

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Despite over 2 million papers published on cancer so far, malignancy still remains a puzzlingly complex disease with overall low survival rates. Expanding our knowledge of the molecular mechanisms of malignancy and of resistance to therapy is crucial in guiding the successful design of anti-cancer drugs and new point-of-care diagnostics. The up-and-coming microfluidic Lab-on-a-Chip (LOC) technology and micro-total analysis systems (μTAS) are arguably the most promising platforms to address the inherent complexity of cellular systems with massive experimental parallelization and 4D analysis on a single cell level. This review discusses the emerging applications of microfluidic technologies and their advantages for cancer biology and experimental oncology. We also summarize the recent advances in miniaturized systems to study cancer cell microenvironment, cancer cytomics, and real-time (4D) pharmacological screening. Microfabricated systems, such as cell microarrays, together with on-chip label-less cytometry, and micro-sorting technologies, are all highlighted with the view of describing their potential applications in pharmacological screening, drug discovery, and clinical oncology. It is envisaged that microfluidic solutions may well represent the platform of choice for next generation *in vitro* cancer models.

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Introduction

During the past decade, a range of DNA and protein microarray technologies have been developed facilitating rapid progress in the fields of experimental and clinical oncology. Transfer of traditional methods to a microfabricated format provides an avenue to increase both the resolution of analysis as well as sampling throughput, while reducing the cost per assay. Most microfabricated technologies, such as DNA microarrays, provide a static readout of studied variables, and as such they are systems that capture only a snap-shot of the intermittent cellular reactions [1]. Moreover, the majority of widely used microfabricated 'array' technologies lack the capability to monitor living cells in real-time. The very nature of tumor cell biology is, however, based on the dynamic, molecular signaling events, providing malignant cells with the ability to adapt to microenvironmental changes [2] (Box 1). Importantly, multiple switches, compensatory and fail-safe mechanisms, and variable response kinetics following anti-cancer drug treatment, are characteristics of malignant cells. Moreover, cancer microenvironment can be viewed as a heterogenic and dynamically evolving molecular ecosystem where cancer cell responses to therapy are influenced by the short range chemical and physical cell-to-cell communications [2,3]. Such level of complexity, with multiple cell types and variables acting at the same time, requires dynamic investigation of many variables at the same time [1–3].

As stated, microfluidics and the design of micro-total analysis systems (μTAS) is the most promising avenue to address the inherent complexity of cellular systems with massive experimental parallelization and measurements of inter-cellular and intra-cellular dynamics in real-time [4] (Box 1). Microfluidics is uniquely aimed at manipulating liquids in networks of microchannels with dimensions between 1 and 1000 μm. At such ultralow volumes, fluids exhibit different physico-chemical properties, when compared to their behavior at the macro-scale. For example, they are dominated by viscous rather than inertial forces [5]. When fluid flow in microchannels is laminar, mass transport will be dominated by local diffusion rates, providing a technique whereby convective contributions can be negated, and the supply of nutrients, gases, and drugs to cells can be controlled and understood [5]. Further, as the inertial forces are minimal, precise delivery of the reagent, in both time and space, is greatly facilitated (where aliquots of fluid can be delivered to particular positions at controlled timings) [4,5]. Most importantly, however, the dimensions of microfluidic environment are comparable to the intrinsic dimensions of cells and blood vessels. Therefore, gas and drug diffusion rates, shear stress, and even microscale cellular niches can be artificially recreated on chip, mimicking the physiological microenvironment encountered in the human body [6••]. These characteristics make microfluidic solutions particularly suitable for emulating artificial tumor environments [6••]. At the same time, the miniaturization of many integrated microfabricated components on chip

Box 1

Microfluidics—new arena of bioengineering aimed at manipulating liquids and particles at ultralow volumes. Small channels and chambers have a cross-sectional area below 1 mm. The dimensionless parameter called the Reynolds number (R_e) describes the unique physical principles of the fluid in microchannels as a function of the channel geometry, fluid viscosity, and flow rate. As described by the Re, fluid flow in microfluidic channels is laminar and dominated by viscous forces. Importantly, in fluids under the laminar flow all fluid particles move in parallel to the flow direction in contrast to the 3D movement of particles in the macro-scale conditions. Under laminar conditions, the fluid flow has no inertia, enabling the precise dosing of drugs, both spatially and temporally. During laminar flow the solute transport is dominated only by a limited and local diffusion. As such it can be effectively used for spatio-temporal stimulation of cells and drug delivery to restricted subcellular compartments.

Cancer—a collective term of genetic chronic diseases characterized by uncontrolled cell growth (deregulated cell proliferation and/or programmed cell death), invasion (incursion to adjacent tissues), and often metastasis to secondary sites (spread to other organs though blood and lymphatic system). Cancer remains a puzzlingly complex molecular disease. Conventional chemotherapy is still largely ineffective for the treatment of metastatic, advanced tumors. Despite recent progress in some investigational, molecular therapies, 5-year survival rates still remain unacceptably low.

Tumor microenvironment—a collective term describing a heterogenic and dynamically evolving 3D molecular ecosystem in which cancer cells interact with each other, with other (normal) cell types and the extracellular matrix (ECM). Cell–cell, cell–matrix, autocrine, paracrine, and hormonal interactions signaling in such environment are characterized by high levels of spatio-temporal complexity. Moreover such environment usually has complex microperfusion (blood supply) that affects gas and drag diffusion rates. The major events relevant to tumorigenesis such as proliferation, survival, and hematogenous spread of the malignant cells are all dependent on specific combinations of cell types and soluble factors present in the micro-environmental niches. Complex tumor microenvironment is also the major factor that affects efficacy of anti-cancer drug action.

allows greatly reduced equipment and reagent costs, and a high level of automation, which is of paramount importance for high-throughput screening routines [7].

LOC has already been widely heralded as an emerging technology with a multitude of applications in cell biology [4,7]. However, implementation of microfluidic systems in cancer research and clinical cancer diagnostics is still in its infancy. LOC solutions provide unique opportunities for single cell cancer cytomics, improved cancer models, and cell sorting technologies. This review discusses the emerging applications of microfluidic technologies that provide new vistas for experimental oncology and anti-cancer drug discovery.

Cancer microenvironment on chip

Tumor microenvironment is a crucial component of cancer biology (including metastatic potential and drug resistance), containing prognostic and response-predictive information, and providing abundant targeting opportunities [8] (Box 1). The major events relevant in tumorigenesis – proliferation, survival, and hematogenous spread of the malignant cells – are all dependent on specific combinations of cell types and soluble factors present in the micro-environmental niches [6,8,9]. Being a heterogenic and dynamically evolving molecular ecosystem, tumor microenvironment is characterized by high levels of spatio-temporal complexity whereby anti-cancer drugs are exquisitely influenced by short range chemical and physical cell-to-cell communication as well as long-range neuro-hormonal cues [8,9]. Microenvironmental conditions of such complexity are particularly difficult to reconstruct using conventional approaches [8,9]. As a result, current means to model tumor microenvironment are technically challenging, do not offer well-defined highly controllable conditions or flexibility of cancer cell analysis, and are not amenable for high-throughput drug screening procedures. Microfluidic platforms can address many of these limitations and mimic more closely the physiological microenvironment that is normally encountered by cells/tissues in the human body. Microperfusion, gas and drug diffusion rates, shear stress, and cell confinement can all be recreated on chip with a higher degree of similarity to physiological conditions, and thus provide new ways to study transient physiological processes, or mimic tumor microenvironment without the use of animal models [6••].

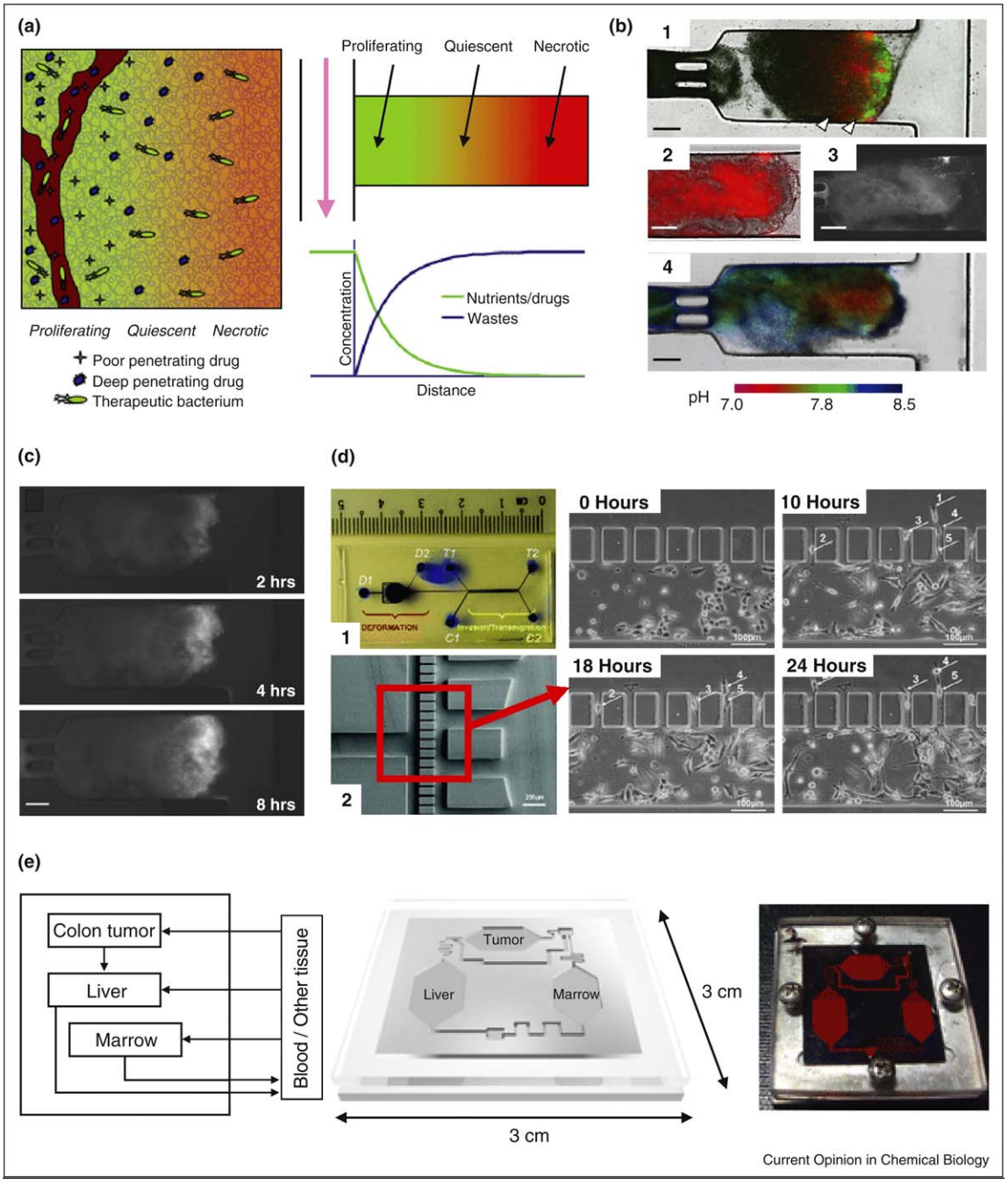
Migration and metastasis

The propensity of cancer cells to extravasate, invade the surrounding tissue and subsequently generate micro and macro metastases to secondary tumor sites is the major cause of mortality in cancer patients [8]. As conventional chemotherapy is still largely ineffective for the treatment of metastatic and advanced tumors, new molecular therapies that target tumor vasculature and metastatic potential are badly needed [8,9]. Until now, however, progress in the field was greatly hampered by a lack of high-throughput and reproducible *in vitro* models of cancer metastasis [10••]. Recently, several microfluidic devices have been proposed for real-time studies of (i) the transmigration and invasion of tumor cells through the artificial endothelial lining/basement membrane barrier [10••]; (ii) invasion of cancer cells into the 3D scaffolds depending on epidermal growth factor (EGF) gradients [11]; (iii) tumor-mimetic culture environments with embedded microvascular structures [12]; and (iv) 3D cancer cell motility in conditions of mechanical confinement in matrix-free environment [13] (Figure 1). New microfluidic technologies that support analysis of cancer cell invasion and metastasis will probably support the development of innovative therapeutic approaches (Figure 1).

Drug distribution and metabolism

Major side effects in the anti-cancer drug development are unforeseen toxicity, inadequate drug penetration into tumor tissue, and the overall lack of target efficacy. The complex and time-dependent processes of drug absorp-

Figure 1



Studies of cancer microenvironment on microscale, Lab-on-a-Chip devices. **(a)** Microenvironment in the microfluidic device mimics the microenvironment surrounding blood vessels in solid tumors. Tumor mass is characterized by nutrient, metabolite, and oxygen gradients in the zones away from blood vessels. This creates varying zones of proliferating (green), quiescent (transition), and necrotic (red) tumor tissue that affect anti-cancer drug penetration, metabolism, and efficacy. Reproduced by permission of The Royal Society of Chemistry from Ref. [6••]. **(b)** Innovative microfluidic device that can mimic the microenvironment gradients in tumor tissue. Note characteristic zones of proliferating, viable (green), and dead (red) cells (panels 1–3). Cellular pH gradient is also evident with acidic and alkaline regions indicated red and blue, respectively (panel 4). Reproduced by permission of The Royal Society of Chemistry from Ref. [6••]. **(c)** Diffusion and penetration of an anti-cancer drug (doxorubicin) into the artificial tumor tissue on the chip described in **(b)** [6••]. **(d)** A multi-step microfluidic device for studying cancer metastasis. A layout of the device with reservoirs, inlet ports for cell seeding, and Matrigel loading ports (left, panel 1). Scanning Electron Micrograph (SEM) of the cell transmigration region (left, panel 2). Cell migration, transmigration, and cell invasion area is 10 mm wide and 150 mm long. Image of MDA-MB-435S cancer cells migrating through the region (red rectangle) shown on panel 2. Device allows for measurements of individual cells' migration rate as well as the population's percentage of cell migration. Reproduced by permission of The Royal Society of Chemistry from Ref. [10••]. **(e)** A micro cell culture analog (μCCA) with 3-D hydrogel culture of multiple cell lines that mimic metabolic functions of different organs and the tumor mass. A complex and time-dependent processes of drug

tion, distribution, metabolism, and elimination (ADME) are difficult to predict using static 2D cell culture systems where drugs are usually applied as a bolus dose to a cell monolayer [14••,15]. Recently, an innovative microfluidic device recreating multi-organ relationships to model pharmacokinetic–pharmacodynamic (PK–PD) interactions has been developed by the group of Michael L. Shuler [14••] (Figure 1). This micro cell culture analog (mCCA) utilizes 3D matrix of hydrogels, forming separate micro-chambers embedded with different cell types that recreate liver, tumor, and bone marrow microenvironments, and are connected by channels that emulate circulating blood flow [14••,15]. Devices of this nature promise a big step towards more physiologically realistic models of tumor microenvironment.

3D cancer models

It is increasingly recognized that cancer cells respond differently to anti-cancer drugs when surrounded by a three-dimensional (3D) extracellular matrix (ECM) than when cultured on a conventional 2D substrate such as polystyrene or glass [16]. Recently an interesting approach to create defined 3D cellular niches on microfluidic devices has been proposed by Huang *et al.* [17••]. Their novel microfluidic device has been utilized with multiple constructs of 3D cell-laden hydrogels and allows for a real-time imaging of cell–cell and cell–matrix interactions exposed to both autocrine and paracrine signaling in a 3D microenvironment [17••] (Figure 1). Other notable developments include the generation of heterotypic 3D multicellular tumor spheroids (MCTS) with fluorescently labeled metastatic prostate cancer cells (PC-3 cell line), osteoblasts, and endothelial cells using a two-layer microfluidic system [18] and microfluidic device that mimics the microenvironment gradients surrounding blood vessels in the tumor mass [6••].

High-throughput drug screening

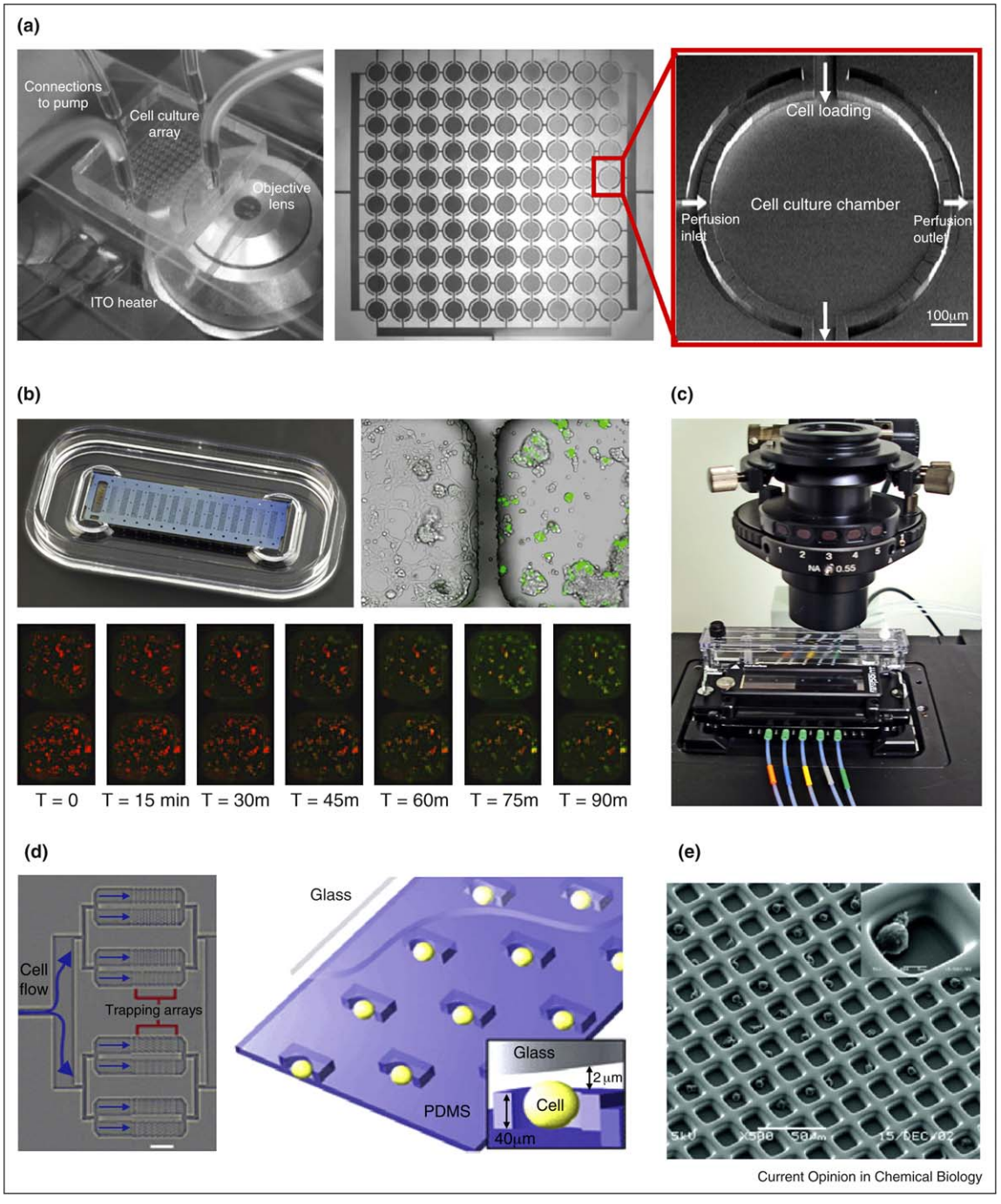
Cost and time savings play an increasing role in anti-cancer drug screening routines. Not surprisingly, enabling strategies that reduce expenditures while increasing throughput and content of information from a given sample are attracting growing interest of the biopharmaceutical community. The development of microfluidic technologies is one of the most innovative approaches towards high-throughput screening with greatly reduced costs, increased sensitivity, and ultra high-throughput by implementing, for example, parallel sample processing [7].

Gradients and integrated cell culture

Laminar flow conditions within microchannels create a unique possibility to generate gradients of drugs and small molecules [4,5]. We have already described that under these circumstances convective mass transport between two co-flowing fluids will be minimal and that diffusion will drive the exchange of materials. Under these conditions, stable gradient of molecules can be produced, whose magnitude vary with distance traveled along the channel (remember in a microfluidic channel under constant flow conditions the spatial position in the channel has a relationship to time). Thus generators and diluters that allow for the automatic generation of controlled drug dilutions can readily be created [19–21]. Several groups have recently proposed different designs that enable long-term culture of cells on chips coupled with a stable gradient of drugs created across the columns of independent microchamber arrays [19–23] (Figure 2). Such systems can support high-throughput, time-resolved analysis of a multitude of diverging cellular outputs (multidimensional/high-content analysis). Of special interest is the high aspect ratio microfluidic device that has been recently designed for the purpose of high-throughput drug screening routines [22]. The main culture chambers of this device are approximately 40 μm in height and 1 mm in diameter, with perfusion performed through an array of small inlet apertures surrounding each microculture chamber (Figure 2). This innovative design greatly improves mass transfer within each chamber, while reducing any adverse effects of shear stress on cultured cells [22]. Another interesting proprietary design has recently been proposed and commercialized by Nanopoint Inc. (Honolulu, Hawaii, USA) under the trademark of CellTRAY® (Figure 2). CellTRAY® consists of independent subregions of glass or plastic microwells that allow for a multiplexed, time-resolved imaging of single cells. Apart from the innovative chip design, Nanopoint Inc. provides also fully integrated and automated control hardware and software that is user-friendly and reportedly can be installed within minutes. Microchip and integrated microfluidics control system can be mounted on an inverted microscope stage and allow for long-term experiments with time-lapse imaging of live cells over the course of several days (Figure 2). We envisage that technologies described here will soon have direct implications for cost and time savings that play an ever increasing role in industrial drug screening pipelines. We anticipate that maturation of such microfluidic technologies in the form of user-friendly and integrated enabling systems will attract a mounting interest from biopharmaceutical and academic communities, and will help to reduce drug development expenditures while increasing throughput and content of information from a given sample [1,7,23] (Figure 2).

(Figure 1 Legend Continued) absorption, distribution, metabolism, and elimination (ADME) can be modeled in real-time on μCCA. This microdevice promises a big step towards more physiologically realistic models of tumor microenvironment. Reproduced by permission of The Royal Society of Chemistry from Ref. [14••].

Figure 2



Cell culture and high-throughput drug screening on microfabricated chips. **(a)** A high aspect ratio microfluidic device for high-throughput drug screening routines. Overview of the device (left panel). Stable gradient generation can be created across the columns of independent microchamber arrays (middle panel). Scanning electron microscope (SEM) image of a single microchamber (right panel). Multiple perfusion channels surround the main culture chamber that is 40 μm in height with a diameter of 1 mm. Reproduced by permission of The Royal Society of Chemistry from Ref. [22]. **(b)** CellTRAY® a novel microfluidic, high-throughput live cell screening technology. Independently addressable regions of glass or plastic microwells allow for a multiplexed and time-resolved experimentation with a single cell resolution. Reproduced with the permission from Nanopoint Inc. (Honolulu, Hawaii, USA). **(c)** Fully integrated and automated CellTRAY® system mounted on a microscope stage. On-microscope incubator and integrated microfluidics system allows for long-term experiments with automated, precise time-lapse imaging of live cells over the course of several days. Reproduced with the permission from Nanopoint Inc. (Honolulu, Hawaii, USA). **(d)** Dynamic single cell culture array based on hydrodynamic cell trapping technology (left panel). A 3D diagram of the design and mechanism of hydrodynamic cell trapping (right panel). Traps are fabricated in PDMS and bonded to a glass substrate. They allow a gentle trapping of single live cells for prolonged periods of time. Reproduced by permission of The Royal Society of Chemistry from Ref. [39]. **(e)** Single cell microarray platform fabricated by patterning PEG (poly(ethylene glycol) diacrylate) hydrogels on

Microflow cytometry

Flow cytometry (FCM) is a powerful analytical and diagnostic tool that leverages the multiparameter and high-speed measurements of cells focused to flow in a single file [1]. It suffers however from high cost, complex operation, and limited portability. Application of microfluidic technology can supersede many of the FCM disadvantages through the development of innovative microflow cytometers (μFCM) that sample a greatly reduced number of cells [24,25] (Figure 3). Many integrated microflow cytometers have recently been proposed, including systems that focus cells into single file using both 2D and 3D hydrodynamic, acoustic, or magnetic focusing [24,25].

Innovative sheath-free cytometers that create ordered streams of cells have also been reported [26,27]. Microfluidic chip-based cytometry is slowly entering a commercial stage with appearance of user-friendly, reasonably priced, and portable devices capable of multiparameter fluorescent interrogation such as CellLab Chip (Agilent Technologies, Santa Clara, CA, USA) or Fishman-R (On-chip biotechnologies Co., Tokyo, Japan) (Figure 3). They are particularly attractive for the clinical and diagnostic laboratories as they allow rapid analysis of only small amounts of patient derived cells.

Label-free microfluidic cytometry

Although flow cytometry is traditionally restricted to interrogating fluorescence markers, miniature label-free cell analysis systems have an immense potential to interrogate intact cells. Spectral impedance measurements using the Coulter principle are an example of label-free on chip cytometry to study cell size as a function of cytoplasmic resistance and membrane capacitance [28]. Recent reports suggest, however, that much more data on cell physiology can be obtained using in-flow capacitance and alternating current (AC) impedance cytometry, and capacitance cytometry [29•,30]. Several innovative cytometric assays have already been developed for the label-free enumeration of antigenically defined cell subpopulations, as well as for analysis of cell cycle and apoptotic changes in composition of plasma membrane phospholipids [29•,30].

Microelectronic alternating current impedance spectroscopy has also been recently developed and commercialized by *Roche Applied Science* in partnership with *ACEA Bioscience* under the trademark of xCELLigence system. It allows label-free analysis of adherent cells cultured on proprietary microelectrode E-plates [31]. It relies on the local ionic environment at the electrode–solution interface, which affects the impedance value of the electrode. Advances have also been made towards microfluidic coherent anti-Stokes Raman scattering (CARS) cytometry [32]. Further development and incorporation of CARS into commercial microfluidic flow cytometers is still some way away (mainly owing to its cost), but in the future, this may facilitate high-throughput cell population analysis coupled with molecular imaging selectivity. Moreover, a number of unconventional cytometric technologies have recently been proposed for a non-invasive and real-time cell analysis on microfluidic chips. These include real-time studies on a single cell level such as time-of-flight (TOF) optophoresis exploiting the moving optical gradient fields for label-less analysis of cells and particles [33]. Using TOF in microfluidic chip, for instance, Zhang *et al.* have been able to discriminate between normal skin and melanoma cell lines and track differentiation of human promyelocytic leukemia HL60 cell line without the need for any fluorescent labels [33]. The application of moving optical gradient fields for the analysis of tumor cell death has also been demonstrated [34].

Single cancer cell cytomics

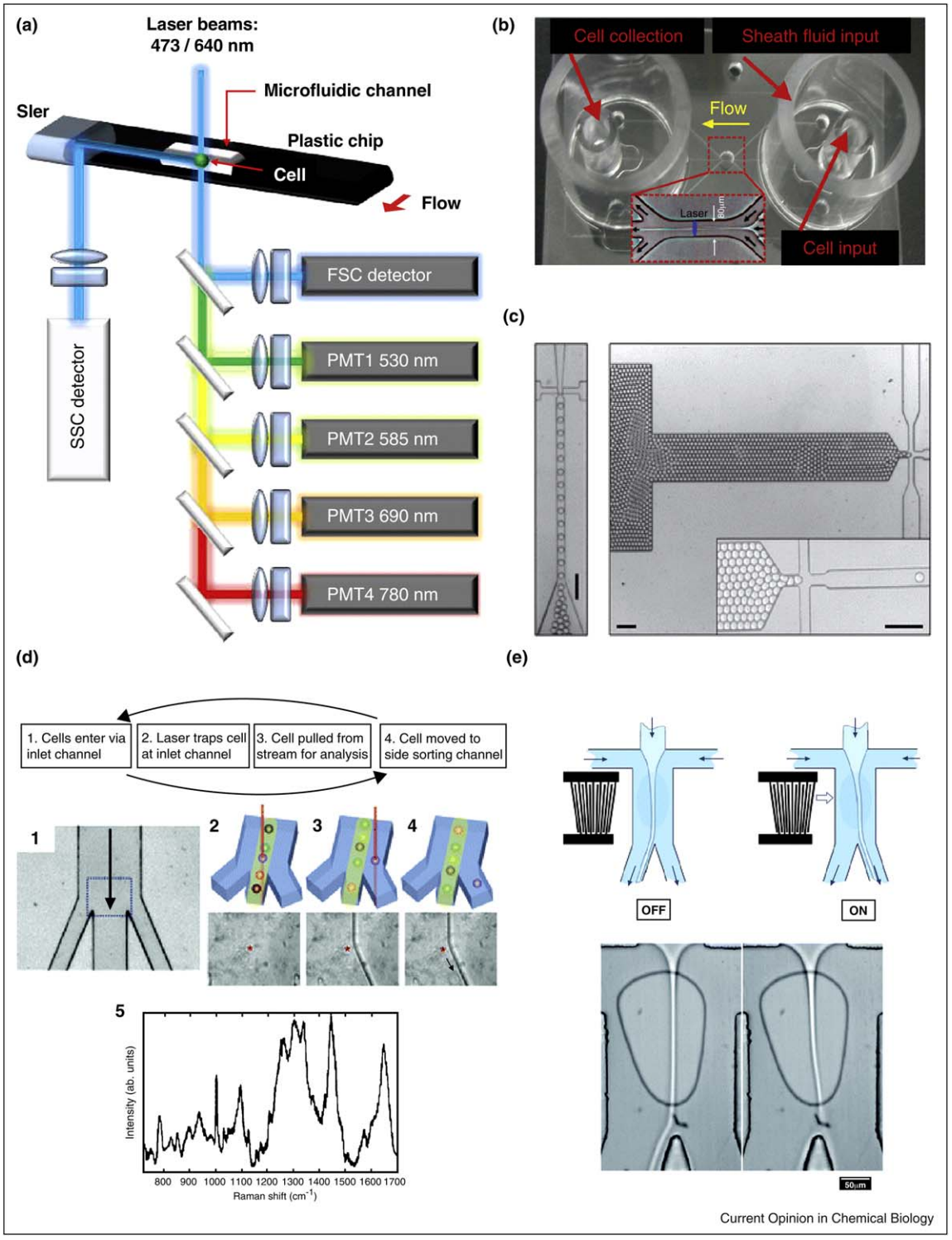
Cancer cell populations represent intrinsically heterogenic and stochastic systems characterized by high levels of spatio-temporal complexity [35]. Multiple variables often act at the same time within such heterogenic ecosystems, providing adaptive and compensatory outcomes that vastly affect cell population structures and thus responses to therapy [35]. Recent mathematical models provided new evidence that intrinsic stochastic variability of biochemical reactions within the cell signaling pathways contributes to the observed cell-to-cell variability in cancer cell survival [36]. Therefore, tracking and understanding cancer cell-to-cell variability emerges as vital to the understanding of cancer cell responses to therapy (Box 1). Microfluidics offers many innovative avenues for single cell cytomics, including microflow cytometry, living-cell microarrays as well as single cell metabolomics.

Micro-NMR

Rapid detection and characterization of primary cancer cells using portable devices is a growing arena for clinical oncology. In this context, a new class of innovative microfabricated magnetic resonance sensors, combining a microfluidic device and a miniaturized NMR probe for detection and molecular profiling of cancer cells, has recently been developed [37••]. This micro-NMR sensor is capable of detecting as few as 2 cancer cells in 1 μl of unprocessed fine-needle aspirates. Such remarkable sensitivity is achieved by measuring the transverse relaxation rate of water molecules in cancer samples

(Figure 2 Legend Continued) glass. Scanning electron micrograph (SEM) shows image of MOLT-3 leukemic cells confined in 15 mm × 15 mm PEG wells. Reproduced by permission of The Royal Society of Chemistry from Ref. [58].

Figure 3



Microfluidic flow cytometers and cell sorters. **(a)** Microfluidic flow cytometer Fishman-R with a multiparameter detection capabilities comparable to the conventional flow cytometers. Note Forward Scatter (FSC), Side Scatter (SSC) and four fluorescence detectors used in combination with spatially separated solid state 473 nm and 640 nm lasers. Side scatter detection is performed using innovative SLER technology (Side scattered Light detection using Edge Reflection) developed by On-chip biotechnologies Co. (Tokyo, Japan) [51,52]. **(b)** Disposable microfluidic cartridge of the Fishman-R microfluidic flow cytometer with a two-way 2D hydrodynamic focusing of cells. Data courtesy of On-chip biotechnologies Co. (Tokyo, Japan) [51,52]. **(c)** Fluorescence-activated droplet sorting (FADS). Generation of a monodisperse emulsion in a microfluidic flow-focusing device produces 12 pl aqueous droplets in fluorinated oil that contains surfactant (left panel). Monodisperse emulsion containing cells can be recovered and stored off chip.

where cells are pre-labeled with magnetic nanoparticles [37••].

Cell microarrays

Cell microarrays and microfluidic cell arrays are other examples of emerging Lab-on-a-Chip technologies that provide important technological advances in the spatio-temporal control of biomolecules and cells [38,39,40•] (Figure 2). They provide innovative ways to simultaneously analyze large population of living cells whereby the position of every cell is encoded and spatially maintained over extended periods of time [38,39,40•]. The main advantage of positioned cell arrays lies in the ability to study the kinetic multivariate signaling events on a single cell level [38,39,40•], which is particularly useful for analysis of cell-to-cell variability and its relevance to cancer therapy. In this context, recent studies in systems oncology have recently revealed new aspects that underlay molecular mechanisms of cell-to-cell variability in cancer cell decision making [2,35,36]. To uncover the stochastic basis of cellular decision making each cell has to be isolated from others to minimize the influence of extrinsic factors such as cell-to-cell contacts and paracrine signaling [2]. LOC systems provide here innovative ways to rapidly analyze large populations whereby the position of every cell is encoded and spatially maintained over the course of experiment. Microfabricated technologies are at present the only tools that can track single cell responses on a large scale.

Many single cell immobilization designs have recently been explored that include static microwells, dielectrophoresis (DEP), as well as micro-mechanical, chemical, and hydrodynamic cell trapping [38,39,40•] (Figure 2). Cells can be reportedly grown in microwells with sizes of the order 10–50 μm and many different designs have been proposed that support large-scale single-cell trapping and extended live cell imaging using static microwell arrays [38] (Figure 2). These chip-based systems are often fabricated in glass or other biocompatible polymers such as poly(dimethylsiloxane) or poly(ethylene glycol) diacrylate (PEG) hydrogels [38]. The passive confinement of cells is achieved by a gravitational sedimentation where cells fall inside microwells and cannot be dislodged. This is a very promising technique mostly owing to its simplicity of fabrication and straightforward operation [38].

More recently the noteworthy reports by groups of Lee, Wikswo, and Cooper have proposed hydrodynamic positioning and immobilization of single cells in arrays of micro-mechanical traps, designed to passively cage individual non-adherent cells (Figure 2) [38,39,40•]. They reportedly allow for rapid trapping of cells in low shear stress zones while being continuously perfused with drugs and sensors [39,40•]. The density and cell trapping efficiency can be easily regulated by changing the number and geometry of microjails. These emerging techniques create a living-cell and dynamic arrays that are ideal for modeling cellular microenvironment and inherently scalable for constructing high-throughput screening platform and future personalized diagnostics [39,40•].

Cells in droplets

An innovative way to study single cells in high-throughput is to encase them inside microfluidic droplets, a technology variously referred to as either segmented flow or micro-emulsion techniques [41••]. In these cases, microfluidic systems allow rapid generation of monodisperse aqueous microdroplets (1 pl to 10 nl in volume) in immiscible carriers, such as oil [41••,42,43]. Because each water droplet is separated from the remaining ones by a layer of oil, it forms a picoliter scale bioreactor. These can be digitally manipulated and used for a wide range of biochemical assays [41••,42,43]. Indeed cell encapsulation and manipulation at a very high-throughput have recently been introduced to droplet-based microfluidics [42,43]. Recent work by Monpichar Srisa-Art and colleagues also showed that fluorescence detection of single cells encapsulated within picoliter-sized aqueous droplets can be performed with a high sensitivity [42,43]. This work provides a new concept for studies of rare phenotypes within heterogeneous populations of cells [42,43].

Another recent innovation in droplet-based microfluidics involves optical traps for spatial and temporal control of cells [44]. In this context, Lanigan *et al.* have developed optically trapped lipid coated oil droplets (Smart Droplet Microtools; SDMs) that are to form membrane tethers upon fusion with the cell plasma membrane [44]. Authors observed that transfer of material, as tracked by membrane localized enhanced green fluorescent protein (EGFP), from the cell membrane to the droplet environment occurred via the tethers [44]. Moreover, SDMs were

(Figure 3 Legend Continued) Monodisperse emulsion can be then transferred to the microfluidic sorting (right panel). AC electric field across the electrodes (1–1.4 kV_{p-p}) is used to deflect droplets into microfluidic channel with a higher hydraulic resistance. Non-deflected droplets flow natively to channel of lower hydraulic resistance. Reproduced by permission of The Royal Society of Chemistry from Ref. [54]. **(d)** Innovative optofluidic Raman-activated cell sorter (RACS). The device combines a laser tweezers Raman spectroscopy (LTRS) with a multichannel microfluidic device for delivery, identification, and sorting of individual cells based on Raman spectra. The diagram describes the RACS operation. The box on panel 1 marks the area in which the LTRS trap is positioned while the arrow shows the direction of the fluid flow. Panels 2–4 illustrate three major steps during the Raman analysis and sorting process (see diagram for description). Panel 5 shows a representative Raman spectrum obtained from a single cell. Reproduced by permission of The Royal Society of Chemistry from Ref. [53]. **(e)** Surface acoustic wave actuated cell sorting (SAWACS). The main stream (top inlet) is hydrodynamically focused by two adjusting side channels. Without surface acoustic wave (SAW) actuation the fluid stream is directed into the left channel because of its lower hydrodynamic resistance. After engaging SAW, acoustic streaming is induced. This deflects the stream into the right channel outlet. The dark blue schematics denotes PDMS substratum whereas black region represents a piezo-electric actuator. Reproduced by permission of The Royal Society of Chemistry from Ref. [55].

able to differentially solubilize membranes of single cells with high spatial selectivity that opens up new opportunities for spatial and temporal manipulation of cell microenvironment [44].

Single cell manipulation

The application of laminar fluid flow under low Reynolds number regimes provides an attractive analytical avenue for the rapid delivery and exchange of reagents to cells [5,45]. By adjusting the fluid flow rate (amount of fluid introduced) the interface between adjacent laminar streams can be precisely controlled and discrete surface regions of the cell can be separately subjected to diverse chemical stimuli [45]. Several successful attempts aimed at single-cell, spatio-temporal stimulation have recently been reported and include studies on intracellular calcium transients, mitochondrial movement, cytoskeleton remodeling, and lateral propagation of epidermal growth factor receptor (EGFR) signaling [45,46]. Other up-and-coming technologies allow for monitoring of single cell behavior upon reversible environmental perturbations using microfluidic chips combined with optical tweezers, acoustic tweezers (standing surface acoustic wave; SSAW technology), and dielectrophoresis [47–49].

Divided and conquered: miniaturized cell sorters

The accurate separation of defined cell subpopulations is of paramount importance for cancer cell biology, clinical diagnostics, and patient-tailored therapies. Not surprisingly, there is an increasing interest and demand for cost-effective and portable cell sorting systems that will supplement conventional flow cytometry especially in: (i) high-throughput cell separation during drug screening routines, (ii) clinical grade cell sorting, (iii) clinical diagnostics, and (iv) exobiology [1,50•,51]. Numerous emerging microfluidic cell sorting devices have been designed, and are based on various cell sorting principles such as piezoelectrical actuation, dielectrophoresis, magnetophoresis, optical gradient forces, and hydrodynamic flow switching (Figure 3).

μFACS

Conventional flow cytometry still remains the technology of choice for rapid quantification and cell separation using high-speed fluorescently activated cell sorting (FACS) [1]. The widespread use of FACS is, nevertheless, severely limited because of its high complexity, power consumption, and resulting intrinsic cost of the equipment and specialized training. This restricts such equipment to only centralized core facilities [1,51,52]. Microfluidic fluorescently activated cell sorters (μFACS) have been proposed, but they mostly suffer from low throughput, lack of integrated mechanisms for actuation, poor sensitivity of fluorescence detection, and low sorting accuracy [50•]. A recent innovation in μFACS, which supersedes most of these problems, is the development of a fully integrated and automated micro-sorter. This design includes microfluidics, as well as optical, acoustical, and piezoelectronic components, all embedded into a single chip, and is further boosted by new automated sorting control systems and signal processing algorithms ('space-time coding technology') that permit high speed sorting of particles combined with high purification enrichment factor [50•]. Other innovative μFACS systems have already been commercialized. The most notable example is the Gigasort™ Clinical Grade Cell Sorter (CytonomeST LLC, Boston, MA, USA), which employs enclosed, disposable chip sorting cartridges, enabling clinical grade, sterile sorting without undesired aerosol formation usually associated with conventional FACS. The Gigasort technology provides also ultra high sorting speed of up to 1 billion events per hour, achieved by a massive parallel array of up to 144 concurrently operating microfluidic sorters (CytonomeST LLC, Boston, MA, USA).

μRACS

Separating individual cells based on their Raman spectral fingerprint is an interesting innovation that moves cell sorting towards label-free chemical cytometry [53] (Figure 3). An integrated device that combines multichannel microfluidic devices with Raman laser tweezers for delivery, identification, and sorting of individual cells has recently been reported by Lau *et al.* [53]. This proof-of-concept study supports the notion that an optofluidics RACS platform can automatically separate individual cancer cells from their normal counterparts based solely on the non-resonant Raman spectroscopic signature (Figure 3).

μFADS and SAWACS

Compartmentalization of single cells into emulsion droplets emerges as a pioneering bioanalytical technology with a plethora of applications in high-throughput pharmacological screening. Building on from this technology, Baret *et al.* have developed a microfluidic fluorescence-activated droplet sorter (FADS) [54] (Figure 3). Single cells are compartmentalized in emulsion droplets that are subsequently sorted using dielectrophoresis in a fluorescence-activated manner. FADS sorting can reportedly achieve rates of up to 2000 droplets per second, while enabling single cell recovery [54]. Another emerging microfluidic cell sorting solution is based on a surface acoustic wave technology [55]. Surface acoustic wave actuated cell sorting (SAWACS) operates in continuous flow at high sorting rates and combines the advantages of both the fluorescence-activated cell sorting and fluorescence-activated droplet sorting [55]. Single cells can be directly sorted using SAWACS at rates as fast as several kHz without prior encapsulation into liquid droplet compartments. Importantly, low shear forces associated with this method ensure cell survival during the sorting procedures, and their subsequent use for other bioassays [55].

Micro-pillar cell sorters

Majority of microfluidic cell sorters robustly manipulate microliter amounts of liquids and small numbers of cells, but they have limited capability to process large volumes of, for example, whole blood samples in a clinical setup [56]. Recently, an interesting sorter design based on a micro-posts array technology has been shown to isolate rare circulating tumor cells from large volume whole blood samples [56,57••]. Cancer cells passing though the device were captured on an array of microdots coated with anti-epithelial-cell-adhesion-molecule (EpCAM; TACSTD1) [54]. Reportedly, this approach allows isolation of extremely rare cell subpopulations (1 target cell in 1 billion blood cells) and is thus ideally suited for real-time monitoring of response to cancer therapy [56,57••].

Conclusions and future trends

Lab-on-a-Chip devices slowly emerge as new platforms with extensive applications in cancer biology, clinical oncology, and high-throughput drug screening routines. Microfluidic devices greatly reduce operational reagent volumes and provide unique opportunities for multiparameter studies on isolated single cancer cells. These are, thus, particularly attractive for the clinical and diagnostic laboratories, allowing rapid analysis of only small amounts of patient derived cells. Moreover, microfluidic technology can provide new vistas for high-throughput reconstruction of cancer microenvironment. It is of great hope that such technologies will support the use of integrated cancer *cytomics* in clinical diagnostics and drug screening routines.

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