WJCO World Journal of Clinical Oncology

Online Submissions: http://www.wjgnet.com/2218-4333office
wjco@wjgnet.com
doi:10.5306/wjco.v1.i1.18

World J Clin Oncol 2010 November 10; 1(1): 18-23
ISSN 2218-4333 (online)
© 2010 Baishideng. All rights reserved.

TOPIC HIGHLIGHT

Joanna Skommer, PhD, *Series Editor*

Microfluidics: Emerging prospects for anti-cancer drug screening

Donald Wlodkowic, Zbigniew Darzynkiewicz

Donald Wlodkowic, Auckland Microfabrication Facility, Department of Chemistry, University of Auckland, 1142 Auckland, New Zealand
Zbigniew Darzynkiewicz, Brander Cancer Research Institute, Department of Pathology, New York Medical College, Valhalla, NY 10595, United States
Author contributions: Wlodkowic D and Darzynkiewicz Z contributed to the design and wrote the manuscript.
Correspondence to: Dr. Donald Wlodkowic, MSc, PhD, Assistant Professor, Auckland Microfabrication Facility, Department of Chemistry, University of Auckland, 23 Symonds Street, 1142 Auckland, New Zealand. d.wlodkowic@auckland.ac.nz
Telephone: +64-9-3737599 Fax: +64-9-3737422
Received: April 21, 2010 Revised: July 27, 2010
Accepted: August 3, 2010
Published online: November 10, 2010

Abstract

Cancer constitutes a heterogenic cellular system with a high level of spatio-temporal complexity. Recent discoveries by systems biologists have provided emerging evidence that cellular responses to anti-cancer modalities are stochastic in nature. To uncover the intricacies of cell-to-cell variability and its relevance to cancer therapy, new analytical screening technologies are needed. The last decade has brought forth spectacular innovations in the field of cytometry and single cell cytomics, opening new avenues for systems oncology and high-throughput real-time drug screening routines. 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. The vast miniaturization of LOC systems and multiplexing enables innovative strategies to reduce drug screening expenditures while increasing throughput and content of information from a given sample. Small cell numbers and operational reagent volumes are sufficient for microfluidic analyzers and, as such, they enable next generation high-throughput and high-content screening of anti-cancer drugs on patient-derived specimens. Herein we highlight the selected advancements in this emerging field of bioengineering, and provide a snapshot of developments with relevance to anti-cancer drug screening routines.

© 2010 Baishideng. All rights reserved.

Key words: Microfluidics; Lab-on-a-chip; Cytometry; Cytomics; Cancer; Anti-cancer drugs; Cancer therapy; Drug screening

Peer reviewers: Des R Richardson, BSc, MSc, PhD, DSc, Professor of Cancer Cell Biology, NHMRC Senior Principal Research Fellow, Director, Iron Metabolism and Chelation Program, Department of Pathology, University of Sydney, Sydney, NSW 2006, Australia; E YK Ng, PhD, PGDTHE, Associate Professor, School of Mechanical and Aerospace Engineering, College of Engineering, Nanyang Technological University, 50, Nanyang Avenue, Singapore 639798, Singapore; Shufeng Zhou, MD, PhD, A/Professor, School of Health Sciences, RMIT University, Bundoora, Victoria 3083, Australia

Wlodkowic D, Darzynkiewicz Z. Microfluidics: Emerging prospects for anti-cancer drug screening. *World J Clin Oncol* 2010; 1(1): 18-23 Available from: URL: http://www.wjgnet.com/2218-4333/full/v1/i1/18.htm DOI: http://dx.doi.org/10.5306/wjco.v1.i1.18

INTRODUCTION

Validation of potential therapeutic targets in cancer requires the introduction of functional live cell assays that provide both spatial and temporal inter-relationships in signaling networks[1,2]. Many cell signaling pathways are initiated and executed through multiple interconnected signaling cascades that differ in both space and time with-

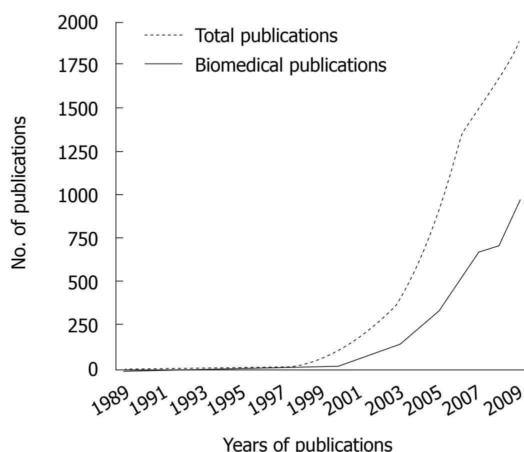

Figure 1 Explosive growth of the microfluidic Lab-on-a-Chip technologies. Note that the last decade brought a dramatic increase in the development of innovative microfabricated technologies aimed at studies of cells and biomolecules. These technologies attract a growing interest within the biomedical community, reflected by a logarithmic growth of publications on the subject. The Scopus database containing citations, abstracts and references covering 16000 peer-reviewed titles from 4000 publishers was used to perform a bibliographical analysis spanning the period between 1989 and 2009. Study was based on the key word "microfluidic" and included two sets of variables: (dashed line) Total publications (database search from "Biology, Agricultural and Environmental Sciences, Chemistry, Physics, Mathematics and Engineering; Life and Health Sciences" collections), (solid line) Biomedical publications (database search from "Biology and Life and Health Sciences" collections). Analysis included only Articles and Reviews and excluded "In press" articles.

in the same cell population encountering the stimulant or drug[3,4]. Modulation of different signaling pathways can lead to additive, synergistic or antagonistic drug actions. Such levels of complexity, with multiple variables acting at the same time, requires an in-depth investigation of cell populations in real-time at a single cell level[1,5,6]. In this context, functional cytomics is slowly becoming an omnipotent part of the post-genomic drug discovery pipeline[7,8]. Although, it is widely recognized that the validation of therapeutic targets revealed by proteomic and genetic screens requires 4D (3D space plus time) functional cell-based assays, their widespread applications are still underdeveloped[1,9,10]. High-content analysis (HCA) is one of the key platforms that recently improved drug screening routines by collecting content-rich data sets[3]. Surprisingly, however, the commonly used HCA approaches are still based on a static principle, yielding information on cell status at a single time point[1,9,10]. Capabilities of high-speed, multiparameter and real-time analysis of great numbers of isolated cells are as yet profoundly limited[1,9,10]. It is still challenging to record, in high-throughput, time-resolved data on a multitude of diverging cellular outputs.

On the other hand, the cost and time savings play an ever increasing role in industrial perspective drug screening routines[3,11]. Not surprisingly, enabling strategies that reduce expenditures, while increasing throughput and content of information from a given sample, attract mounting interest within the biopharmaceutical community. The last decade has brought many innovations to the field of cytomics and cytometry[10]. Probably the most fascinating are the prospects and explosive development of innovative micro- and nanofluidic Lab-on-a-Chip (LOC) technologies (Figure 1)[10-15]. Transfer of traditional bioanalytical methods to a microfabricated format provides the means to increase both the resolution of analysis and sampling throughput while reducing the cost of a single assay[10-15]. By providing an alternative to expensive instrumentation, such as flow or laser scanning cytometers and sorters, which are unaffordable for small research or clinical laboratories, these miniaturized tools of cytometry can be used more widely and also can be available in underprivileged countries.

EMERGING PROSPECT OF MICROFLUIDICS FOR CANCER RESEARCH

Microfluidics is a new arena of bioengineering aimed at manipulating liquids and particles in ultralow volumes in small channels that have a cross-sectional area less than a square millimeter (mm^2)[12,16,17]. The dimensionless parameter, called the Reynolds number (R_e), describes unique physical principles of the fluid in microchannels as a function of the channel geometry, fluid viscosity and flow rate (Figure 2)[16,17]. As described by the R_e, fluid flow in microfluidic channels is laminar and dominated by viscous forces (Figure 2). Importantly, in fluids under laminar flow, all fluid particles move in parallel to the flow direction in contrast to the 3D movement of particles in macroscale conditions (Figure 2)[16,17]. Under laminar conditions, the fluid flow has no inertia, enabling the precise dosing of drugs, both spatially and temporally[16,17]. Moreover, during laminar flow, the solute transport is dominated by a limited and local diffusion (Figure 2)[16,17]. As such, it can be effectively used for spatio-temporal stimulation of cells and drug delivery to restricted subcellular compartments (Figure 2)[18,19].

The enclosed and sterile formats of microfluidic LOC devices eliminate evaporative water loss from microsized channels[20]. Biocompatible and inexpensive polymers, such as polydimethylsiloxane (PDMS), are often materials of choice for the fabrication of disposable cell-based microfluidic devices[20,21]. These innovative polymers prevent cross-contamination of biological specimens that are subjected to parallel stimulation with a number of different drugs[20,21]. Innovative biopolymers used for the fabrication of microfluidic devices also provide secure biocontainment of infectious specimens, such as viral gene vectors or HIV^+ and blood samples[20,21]. When desirable, current microfabrication techniques also allow the creation of microfluidic circuitry in glass and quartz, which enhances the durability and application range in high-pressure applications.

Undoubtedly, the advent of microfluidics, and its integration into the design of micro-total analysis systems (μTAS), is leading to one of the most adventurous avenues to address the inherent complexity of cellular systems, with unprecedented experimental high-throughput at the single cell level (Figure 3)[10-17]. While the application of laminar fluid flow under low Reynolds numbers pro-

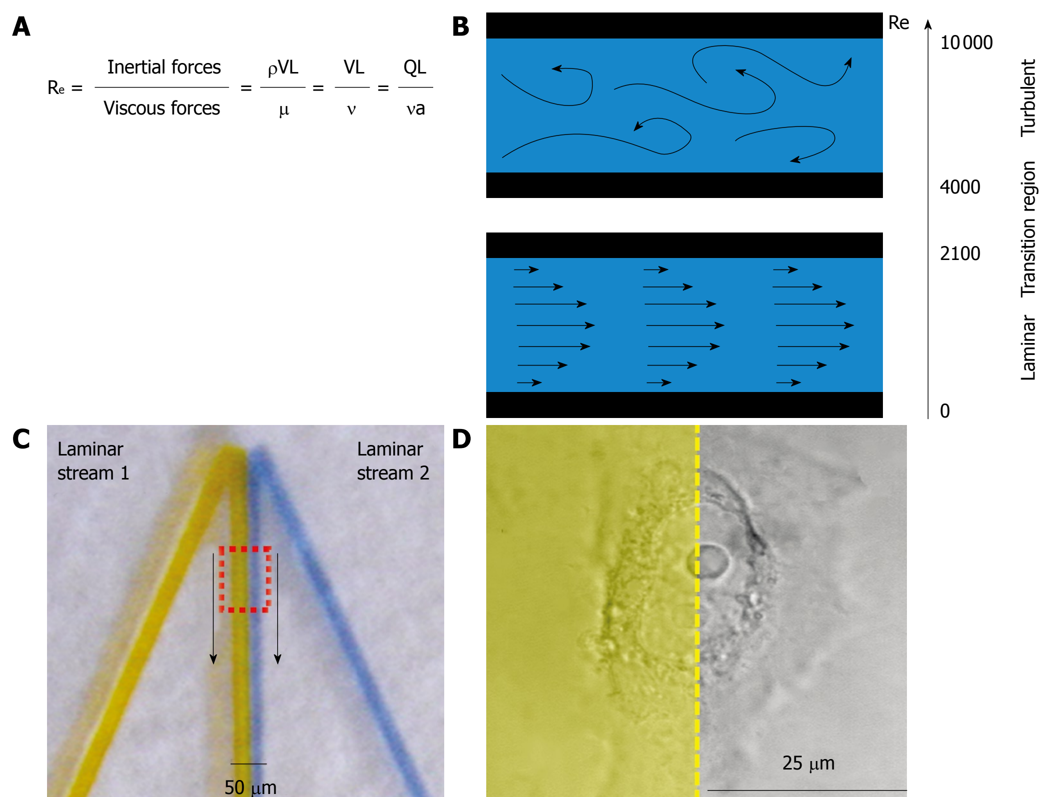

Figure 2 Principles of microfluidics. A: Microfluidics is aimed at manipulating liquids at ultralow volumes. The dimensionless parameter, the Reynolds number (R_e), describes unique physical principles of the fluid in channels within a cross-sectional area as a function of the channel geometry, fluid viscosity and flow rate. R_e is the measure of the ratio between the inertial to viscous forces where: ρ: density of the fluid (kg/m^3); V: Mean fluid velocity (m/s); L: Length of the channel (m); μ: Dynamic fluid viscosity (Pa•s); ν: Kinematic fluid viscosity (m^2/s); Q: Volumetric flow rate (m^3/s); a: Cross-sectional area of the channel (m^2); B: As described by the R_e, fluid flow in microfluidic channels is dominated by viscous rather than inertial forces. Laminar flow describes the conditions where all fluid particles move in parallel to the flow direction. Laminar flow is therefore represented by R_e values below 2100. In contrast, turbulent flow is characterized by movement of fluid particles in all three dimensions that do not correlate with the overall direction of the fluid flow. Turbulent flow is thus represented by larger R_e values (above 4000). R_e values between 2100 and 4000 describe the transition region where fluid flow may have the features of both laminar and turbulent flow; C: Laminar flow under low R_e can be effectively used for spatiotemporal stimulation of cells. Note that during fluid flow under low Reynolds numbers solute transport is dominated only by limited and local diffusion. Cell positioning is marked in red; D: An example of drug delivery to selected cell compartments using laminar flow streams. Phase-contrast image of HeLa cells stimulated using laminar flow under low Reynolds numbers. The yellow area (laminar stream 1) denotes the restricted cell compartment to which the drug is being delivered using laminar flow (as shown in panel C).

vides an attractive analytical avenue for the rapid delivery and exchange of reagents with exceptional accuracy, the transfer of traditional methods to a microfabricated format offers a means to increase both the resolution of analysis and sampling throughput while reducing the cost of a single assay (Figure 3)[10-17]. The disposable format of many LOC devices is particularly suitable for point-of-care diagnostics and future personalized therapy[22-25]. LOC devices also promise greatly reduced costs, increased sensitivity and ultra high throughput by implementing parallel sample processing and a vast miniaturization of integrated on-chip components (Figure 3)[14,26].

A number of emerging, microfluidic technologies for cell-based assays have recently been reported[13,14]. For instance, microfluidics offers an exceptional evolutionary route for flow cytometry, a technique known as microflow cytometry (μFCM)[27-32]. Micro fluorescently activated cell sorting (μFACS) and in-flow magnetically activated cell sorting (μMACS) are other rapidly up-and-coming examples of high-throughput on-chip cytometric technologies with substantial potential in anti-cancer drug discovery and personalized diagnostics[32-36]. Microfluidic flow cytometers and cell sorters require a greatly reduced number of cells per sample when compared with conventional FACS[27-36].

Spectral impendence using the Coulter principle has also been adapted for on chip devices to study the function of cell size, cytoplasmic resistance and membrane capacitance[37-39]. Precise differential white blood cell counts have already been demonstrated using this approach[40,41]. Recent reports suggest, however, that more high-throughput data can be obtained using in-flow dielectric spectroscopy on chips[42,43]. In this regard, innovative high throughput screening (HTS) technologies that are developed in a miniaturized format include capacitance and impedance cytometry[43-45]. Moreover, a number of unconventional 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 and scanning thermal lens microscopy (TLM)[46-50].

The living cell microarrays and microfluidic cell arrays are yet other examples of emerging LOC technologies that provide important technological advances in the

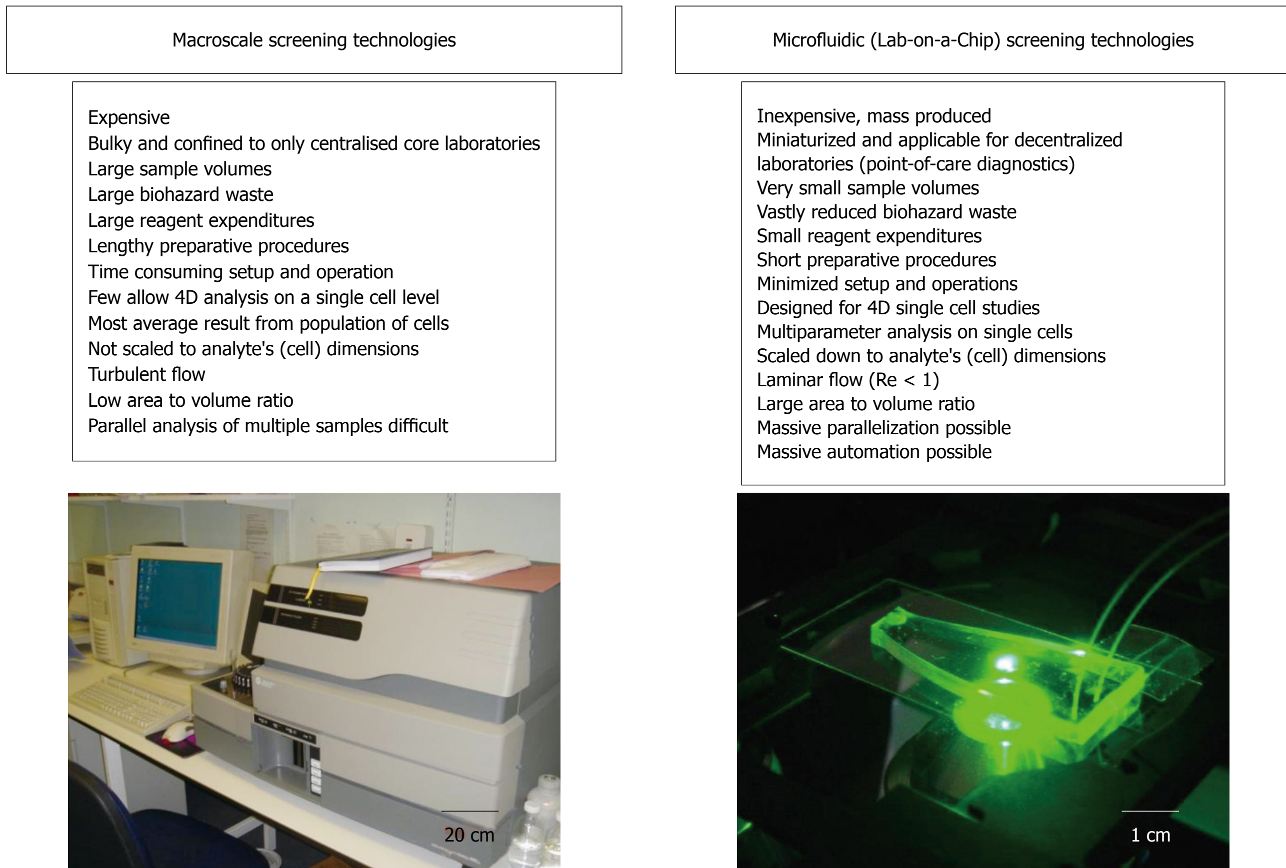

Figure 3 Comparison between conventional (macroscale) and microfluidic (Lab-on-a-Chip) technologies for cell-based assays. The advent of microfluidics and its integration into design micro-total analysis systems (μTAS) and Lab-on-a-Chip (LOC) devices is one of the most promising avenues to address the inherent complexity of cellular systems with massive experimental parallelization and analysis on a single cell level. LOC technologies promise greatly reduced equipment costs, simplified operation, increased sensitivity and throughput by implementing parallel processing principles and a vast miniaturized of on-chip components. Only low cell numbers and operational reagent volumes are required for LOC technology. It, in turn, opens up new prospects for high-throughput and high-content screening of anti-cancer drugs on patient derived specimens.

spatiotemporal control of biomolecules and cells[6,10,51-56]. Pioneering microfluidic cell arrays allow collection of real-time and multiparameter data obtained from functional cell-based assays[6,10,56]. These emerging technologies create living-cell arrays that are ideal for modeling cancer microenvironments and inherently scalable for constructing a high-throughput screening platform[52-55]. Its particular advantage lies in the ability to enable the kinetic and multivariate analysis of signaling events on a single cell level[6,10,55,56]. Cell microarray technology seems, thus, to be particularly suitable for uncovering intricacies in cell-to-cell variability and its relevance to cancer therapy. Recent studies in systems biology have recently shed new light on the underlying molecular mechanisms of cell-to-cell variability in cancer cell decision making[57,58]. 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. LOC systems provide innovative ways to simultaneously analyze large populations of cells whereby the position of every cell is encoded and spatially maintained over extended periods of time[6,54,56]. In this regard, microfluidic platforms that can track single cell responses on a large scale are, thus far, the only tool that can support the integrative mathematical oncology with systems biology efforts to yield new vistas for a generation of rationally designed anti-cancer drugs. Our recent studies have validated the application of live-cell microarrays for the kinetic analysis of investigational anti-cancer agents in hematopoietic cancer cells and hematopoietic cancer stem cells[6,56].

CONCLUSION

Understanding cell-to-cell variability in cancer is fundamental to the development of successful therapeutic regimens[57-59]. In this context, systems biology, cytomics and integrative mathematical oncology are new research arenas that can explain and simulate the cell-to-cell variability in cancer cell responses[57-59]. The experimental confirmation of mathematical models is difficult, however, mainly due to the inherent heterogeneity and complexity of cellular systems. Limitations of conventional cell-based techniques, such as flow cytometry and single cell imaging, make high-throughput dynamic analysis on cellular and subcellular processes tedious and exceedingly expensive[10]. Moreover, conventional assays do not incorporate physiological processes that are normally encountered by cells/tissues

in the human body, such as microperfusion, gas/drug diffusion rates and shear stress[12-15]. These design limitations of macroscale analytical systems have led to a biased understanding of many transient and intermittent physiological processes. This is often reflected by the failure of many therapeutic leads, selected after *in vitro* screening, to perform *in vivo* in animal models[60]. The microfluidic platforms that can track single cell responses, multiparametrically on a large scale are, so far, the only tools that can support mathematical oncology and systems biology efforts and provide new vistas for a new generation of rationally designed anti-cancer drugs. LOC systems provide innovative ways to simultaneously analyze large populations of cells whereby the position of every cell is encoded and spatially maintained over extended periods of time. The microfluidic environment closely mimics the physiological microenvironment, including gas and drug diffusion rates, shear stress and cell confinement. In the context of tumor biology, for example, and anti-cancer drug discovery, microfluidic technologies warrant a "quantum leap" for drug discovery and personalized diagnostics.

REFERENCES

1 **Wlodkowic D**, Skommer J, McGuinness D, Faley S, Kolch W, Darzynkiewicz Z, Cooper JM. Chip-based dynamic real-time quantification of drug-induced cytotoxicity in human tumor cells. *Anal Chem* 2009; **81**: 6952-6959

2 **Tárnok A**, Valet GK, Emmrich F. Systems biology and clinical cytomics: The 10th Leipziger Workshop and the 3rd International Workshop on Slide-Based Cytometry, Leipzig, Germany, April 2005. *Cytometry A* 2006; **69**: 36-40

3 **Mayr LM**, Bojanic D. Novel trends in high-throughput screening. *Curr Opin Pharmacol* 2009; **9**: 580-588

4 **Wlodkowic D**, Skommer J, McGuinness D, Hillier C, Darzynkiewicz Z. ER-Golgi network--a future target for anti-cancer therapy. *Leuk Res* 2009; **33**: 1440-1447

5 **Svahn HA**, van den Berg A. Single cells or large populations? *Lab Chip* 2007; **7**: 544-546

6 **Wlodkowic D**, Faley S, Zagnoni M, Wikswo JP, Cooper JM. Microfluidic single-cell array cytometry for the analysis of tumor apoptosis. *Anal Chem* 2009; **81**: 5517-5523

7 **Tárnok A**, Bocsi J, Brockhoff G. Cytomics - importance of multimodal analysis of cell function and proliferation in oncology. *Cell Prolif* 2006; **39**: 495-505

8 **Wlodkowic D**, Cooper JM. Microfabricated analytical systems for integrated cancer cytomics. *Anal Bioanal Chem* 2010; **398**: 193-209

9 **Zhao H**, Oczos J, Janowski P, Trembecka D, Dobrucki J, Darzynkiewicz Z, Wlodkowic D. Rationale for the real-time and dynamic cell death assays using propidium iodide. *Cytometry A* 2010; **77**: 399-405

10 **Wlodkowic D**, Skommer J, Darzynkiewicz Z. Cytometry in cell necrobiology revisited. Recent advances and new vistas. *Cytometry A* 2010; **77**: 591-606

11 **Schmid EF**, Ashkenazy R, Merson J, Smith DA. Will biomedical innovation change the future of healthcare? *Drug Discov Today* 2009; **14**: 1037-1044

12 **Whitesides GM**. The origins and the future of microfluidics. *Nature* 2006; **442**: 368-373

13 **El-Ali J**, Sorger PK, Jensen KF. Cells on chips. *Nature* 2006; **442**: 403-411

14 **Sims CE**, Allbritton NL. Analysis of single mammalian cells on-chip. *Lab Chip* 2007; **7**: 423-440

15 **Andersson H**, van den Berg A. Microtechnologies and nanotechnologies for single-cell analysis. *Curr Opin Biotechnol* 2004; **15**: 44-49

16 **Squires TM**. Microfluidics: Fluid physics at the nanoliter scale. *Rev Mod Phys* 2005; **77**: 977-1026

17 **Stone HA**, Stroock AD, Ajdari A. Engineering flows in small devices: microfluidics toward a Lab-on-a-Chip. *Annu Rev Fluid Mech* 2004; **36**: 381–411

18 **Takayama S**, Ostuni E, LeDuc P, Naruse K, Ingber DE, Whitesides GM. Subcellular positioning of small molecules. *Nature* 2001; **411**: 1016

19 **Takayama S**, Ostuni E, LeDuc P, Naruse K, Ingber DE, Whitesides GM. Selective chemical treatment of cellular microdomains using multiple laminar streams. *Chem Biol* 2003; **10**: 123-130

20 **Wlodkowic D**, Faley S, Skommer J, McGuinness D, Cooper JM. Biological implications of polymeric microdevices for live cell assays. *Anal Chem* 2009; **81**: 9828-9833

21 **Sia SK**, Whitesides GM. Microfluidic devices fabricated in poly(dimethylsiloxane) for biological studies. *Electrophoresis* 2003; **24**: 3563-3576

22 **Myers FB**, Lee LP. Innovations in optical microfluidic technologies for point-of-care diagnostics. *Lab Chip* 2008; **8**: 2015-2031

23 **Mauk MG**, Ziober BL, Chen Z, Thompson JA, Bau HH. Lab-on-a-chip technologies for oral-based cancer screening and diagnostics: capabilities, issues, and prospects. *Ann N Y Acad Sci* 2007; **1098**: 467-475

24 **Weigl B**, Domingo G, Labarre P, Gerlach J. Towards non- and minimally instrumented, microfluidics-based diagnostic devices. *Lab Chip* 2008; **8**: 1999-2014

25 **Kiechle FL**, Holland CA. Point-of-care testing and molecular diagnostics: miniaturization required. *Clin Lab Med* 2009; **29**: 555-560

26 **Dittrich PS**, Manz A. Lab-on-a-chip: microfluidics in drug discovery. *Nat Rev Drug Discov* 2006; **5**: 210-218

27 **Chan SD**, Luedke G, Valer M, Buhlmann C, Preckel T. Cytometric analysis of protein expression and apoptosis in human primary cells with a novel microfluidic chip-based system. *Cytometry A* 2003; **55**: 119-125

28 **Huh D**, Gu W, Kamotani Y, Grotberg JB, Takayama S. Microfluidics for flow cytometric analysis of cells and particles. *Physiol Meas* 2005; **26**: R73-R98

29 **Wolff A**, Perch-Nielsen IR, Larsen UD, Friis P, Goranovic G, Poulsen CR, Kutter JP, Telleman P. Integrating advanced functionality in a microfabricated high-throughput fluorescent-activated cell sorter. *Lab Chip* 2003; **3**: 22-27

30 **Takeda K**, Jimma F. Maintenance free biosafety flowcytometer using disposable microfluidic chip (FISHMAN-R). *Cytometry B* 2009; **76B**: 405-406

31 **Takao M**, Jimma F, Takeda K. Expanded applications of new-designed microfluidic flow cytometer (FISHMAN-R). *Cytometry B* 2009; **76B**: 405

32 **Wang MM**, Tu E, Raymond DE, Yang JM, Zhang H, Hagen N, Dees B, Mercer EM, Forster AH, Kariv I, Marchand PJ, Butler WF. Microfluidic sorting of mammalian cells by optical force switching. *Nat Biotechnol* 2005; **23**: 83-87

33 **Fu AY**, Chou HP, Spence C, Arnold FH, Quake SR. An integrated microfabricated cell sorter. *Anal Chem* 2002; **74**: 2451-2457

34 **Sugino H**, Ozaki K, Shirasaki Y, Arakawa T, Shoji S, Funatsu T. On-chip microfluidic sorting with fluorescence spectrum detection and multiway separation. *Lab Chip* 2009; **9**: 1254-1260

35 **Adams JD**, Kim U, Soh HT. Multitarget magnetic activated cell sorter. *Proc Natl Acad Sci USA* 2008; **105**: 18165-18170

36 **Pamme N**, Wilhelm C. Continuous sorting of magnetic cells via on-chip free-flow magnetophoresis. *Lab Chip* 2006; **6**: 974-980

37 **Rodriguez-Trujillo R**, Castillo-Fernandez O, Garrido M, Arundell M, Valencia A, Gomila G. High-speed particle

detection in a micro-Coulter counter with two-dimensional adjustable aperture. *Biosens Bioelectron* 2008; **24**: 290-296

38 **Scott R**, Sethu P, Harnett CK. Three-dimensional hydrodynamic focusing in a microfluidic Coulter counter. *Rev Sci Instrum* 2008; **79**: 046104

39 **Zheng S**, Liu M, Tai YC. Micro coulter counters with platinum black electroplated electrodes for human blood cell sensing. *Biomed Microdevices* 2008; **10**: 221-231

40 **Holmes D**, Pettigrew D, Reccius CH, Gwyer JD, van Berkel C, Holloway J, Davies DE, Morgan H. Leukocyte analysis and differentiation using high speed microfluidic single cell impedance cytometry. *Lab Chip* 2009; **9**: 2881-2889

41 **Piacentini N**, Demarchi D, Civera P, Knaflitz M. Blood cell counting by means of impedance measurements in a microsystem device. *Conf Proc IEEE Eng Med Biol Soc* 2008; **2008**: 4824-4827

42 **Wang X**, Becker FF, Gascoyne PR. Membrane dielectric changes indicate induced apoptosis in HL-60 cells more sensitively than surface phosphatidylserine expression or DNA fragmentation. *Biochim Biophys Acta* 2002; **1564**: 412-420

43 **Cheung K**, Gawad S, Renaud P. Impedance spectroscopy flow cytometry: on-chip label-free cell differentiation. *Cytometry A* 2005; **65**: 124-132

44 **Sohn LL**, Saleh OA, Facer GR, Beavis AJ, Allan RS, Notterman DA. Capacitance cytometry: measuring biological cells one by one. *Proc Natl Acad Sci USA* 2000; **97**: 10687-10690

45 **Atienza JM**, Zhu J, Wang X, Xu X, Abassi Y. Dynamic monitoring of cell adhesion and spreading on microelectronic sensor arrays. *J Biomol Screen* 2005; **10**: 795-805

46 **Zhang H**, Tu E, Hagen ND, Schnabel CA, Paliotti MJ, Hoo WS, Nguyen PM, Kohrumel JR, Butler WF, Chachisvillis M, Marchand PJ. Time-of-flight optophoresis analysis of live whole cells in microfluidic channels. *Biomed Microdevices* 2004; **6**: 11-21

47 **Forster AH**, Wang MM, Butler WF, Chachisvilis M, Chung TD, Esener SC, Hall JM, Kibar O, Lykstad K, Marchand PJ, Mercer EM, Pestana LM, Sur S, Tu E, Yang R, Zhang H, Kariv I. Use of moving optical gradient fields for analysis of apoptotic cellular responses in a chronic myeloid leukemia cell model. *Anal Biochem* 2004; **327**: 14-22

48 **Nerenberg M**, Kariv I, McNeeley P, Marchand P, Sur S, Diver J, Riccitelli S, Nieva J, Saven A. Use of optophoresis as an in vitro predictor of cell response to chemotherapy for chronic lymphocytic leukemia. *Leuk Lymphoma* 2006; **47**: 2194-2202

49 **Tamaki E**, Hibara A, Tokeshi M, Kitamori T. Microchannel-assisted thermal-lens spectrometry for microchip analysis. *J Chromatogr A* 2003; **987**: 197-204

50 **Tamaki E**, Hibara A, Tokeshi M, Kitamori T. Tunable thermal lens spectrometry utilizing microchannel-assisted thermal lens spectrometry. *Lab Chip* 2005; **5**: 129-131

51 **Yamamura S**, Kishi H, Tokimitsu Y, Kondo S, Honda R, Rao SR, Omori M, Tamiya E, Muraguchi A. Single-cell microarray for analyzing cellular response. *Anal Chem* 2005; **77**: 8050-8056

52 **Rettig JR**, Folch A. Large-scale single-cell trapping and imaging using microwell arrays. *Anal Chem* 2005; **77**: 5628-5634

53 **Lindström S**, Mori K, Ohashi T, Andersson-Svahn H. A microwell array device with integrated microfluidic components for enhanced single-cell analysis. *Electrophoresis* 2009; **30**: 4166-4171

54 **Di Carlo D**, Wu LY, Lee LP. Dynamic single cell culture array. *Lab Chip* 2006; **6**: 1445-1449

55 **Yarmush ML**, King KR. Living-cell microarrays. *Annu Rev Biomed Eng* 2009; **11**: 235-257

56 **Faley SL**, Copland M, Wlodkowic D, Kolch W, Seale KT, Wikswo JP, Cooper JM. Microfluidic single cell arrays to interrogate signalling dynamics of individual, patient-derived hematopoietic stem cells. *Lab Chip* 2009; **9**: 2659-2664

57 **Lavrik IN**, Eils R, Fricker N, Pforr C, Krammer PH. Understanding apoptosis by systems biology approaches. *Mol Biosyst* 2009; **5**: 1105-1111

58 **Raychaudhuri S**, Skommer J, Henty K, Birch N, Brittain T. Neuroglobin protects nerve cells from apoptosis by inhibiting the intrinsic pathway of cell death. *Apoptosis* 2010; **15**: 401-411

59 **Enderling H**, Anderson AR, Chaplain MA, Beheshti A, Hlatky L, Hahnfeldt P. Paradoxical dependencies of tumor dormancy and progression on basic cell kinetics. *Cancer Res* 2009; **69**: 8814-8821

60 **Rowinsky EK**. Curtailing the high rate of late-stage attrition of investigational therapeutics against unprecedented targets in patients with lung and other malignancies. *Clin Cancer Res* 2004; **10**: 4220s-4226s

S- Editor Cheng JX **L- Editor** Lutze M **E- Editor** Ma WH