

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/26692906>

A self-contained microfluidic cell culture system

Article in *Biomedical Microdevices* · August 2009

DOI: 10.1007/s10544-009-9342-4 · Source: PubMed

CITATIONS

38

READS

374

4 authors, including:



Min-Cheol Kim

Massachusetts Institute of Technology

70 PUBLICATIONS 839 CITATIONS

[SEE PROFILE](#)



Zhanhui Wang

MNChip

16 PUBLICATIONS 455 CITATIONS

[SEE PROFILE](#)

A self-contained microfluidic cell culture system

Baoyue Zhang · Min-Cheol Kim · Todd Thorsen ·
Zhanhui Wang

© Springer Science + Business Media, LLC 2009

Abstract Conventional *in vitro* cell culture that utilizes culture dishes or microtiter plates is labor-intensive and time-consuming, and requires technical expertise and specific facilities to handle cell harvesting, media exchange and cell subculturing procedures. A microfluidic array platform with eight microsieves in each cell culture chamber is presented for continuous cell culture. With the help of the microsieves, uniform cell loading and distribution can be obtained. Within the arrays, cells grown to the point of confluency can be trypsinized and recovered from the device. Cells trapped in the microsieves after trypsinization function to seed the chambers for subsequent on-chip culturing, creating a sustainable platform for multiple cycles. The capability of the microfluidic array platform was demonstrated with a BALB/3T3 (murine embryonic fibroblast) cell line. The present microfluidic cell culture platform has potential to develop into a fully automated cell culture system integrated with temperature control, fluidic control, and micropumps, maximizing cell culture health with minimal intervention.

Keywords Microfluidics · Cell culture · Microsieve

B. Zhang · Z. Wang (✉)
Institute of Biomedical and Health Engineering,
Shenzhen Institutes of Advanced Technology,
Chinese Academy of Sciences,
Shenzhen, China 518067
e-mail: zh.wang@siat.ac.cn

B. Zhang
Beijing Technology and Business University,
Beijing, China 100037

M.-C. Kim · T. Thorsen
Department of Mechanical Engineering,
Massachusetts Institute of Technology,
Cambridge, MA 02139, USA

1 Introduction

In vitro mammalian cell culture is an essential and powerful tool in biological science. It has made substantial contribution to the understanding of many phenomena, such as intracellular enzyme activities, intracellular flux, and cell-cell interactions, since it originated over one century ago. In recent years, cell culture in high-density array formats, including microfluidic arrays, has attracted tremendous interest due to the potential for rapid large scale cell-based assays. Many microfluidic devices have been developed for cell culture (Balagaddé et al. 2005; Hufnagel et al. 2009; Hung and Lee 2007; Petronis et al. 2006; Stangegaard et al. 2006) and to study intracellular enzyme activities (Carlo et al. 2006), cellular responses to chemical gradients (Jeon et al. 2002; Hung et al. 2005), cellular differentiation (Tourovskaya et al. 2005), dynamic gene expression (King et al. 2007) and cell cytotoxicity screening (Wang et al. 2007). Some microfluidic devices were designed for cell culture purpose in a high-throughput manner (Hung et al. 2005; Gomez-Sjoberg et al. 2007), however, few devices can establish a uniform distribution of cells in whole microfluidic cell array.

As the research emphasis shifts from bulk cell culture, where the behavior of tens of thousands of cells are assayed in individual microwells, to research at the single cell level, cell culture viability and standardized, uniform growth conditions become increasingly important. In bulk culture formats, variations in cell seeding density, nutrient delivery and waste removal are sources of stress on cell cultures that introduce intracellular variations in subsequent assay procedures. Moreover, traditional culture methodology requires technical expertise and specific facilities to handle cells harvesting, media exchanging and cell sub-culturing procedures. In this paper, we describe the development of a continuous microfluidic cell culture platform, in which cells

can be repeatedly grown to confluence, trypsinized and recovered. Integrated arrays of U-shaped sieves within round microchambers promote uniform spatial seeding of small clusters of cells ($\sim 10/\text{sieve}$; $\sim 100/\text{chamber}$), while the ability to tightly regulate the media delivery and removal from the chambers promotes cell viability.

2 Materials and methods

2.1 Microfluidic device fabrication

The microfluidic device used for cell culture was fabricated from poly(dimethylsiloxane) PDMS (Sylgard 184, Dow Corning) using replicate molding and soft lithography (Zhao et al. 1997). SU-8 50 (Microchem, USA) was patterned on a silicon wafer using high-resolution design transparencies to create the distinct positive-relief casting mold for the elastomer. The mold was subsequently exposed to vapor phase chloro-trimethylsilane (Aldrich) to facilitate the release of the elastomer during molding. Preparing the elastomer, Sylgard 184 liquid silicone elastomer (mixed in a ratio of 10 part A : 1 part B) was poured on the mold (~ 5 mm thick), and baked at 80°C for 2 h. After baking, the cured PDMS layer was peeled from the mold, fluid inlet and outlet ports were punched using a 23 gauge luer stub (BD Biosciences). To remove residue generated during the punching process, the microchannels and connection ports were cleaned with isopropanol, dried with nitrogen, and the exposed microchannel surface on the molded device was irreversibly bonded to a glass slide using oxygen plasma (150 mTorr, 50 W, 20 s).

2.2 Cell culture

BALB/3T3 murine embryonic fibroblast cells were acquired from Advanced Type Culture Collection (ATCC), and cell culture media and supplements were purchased

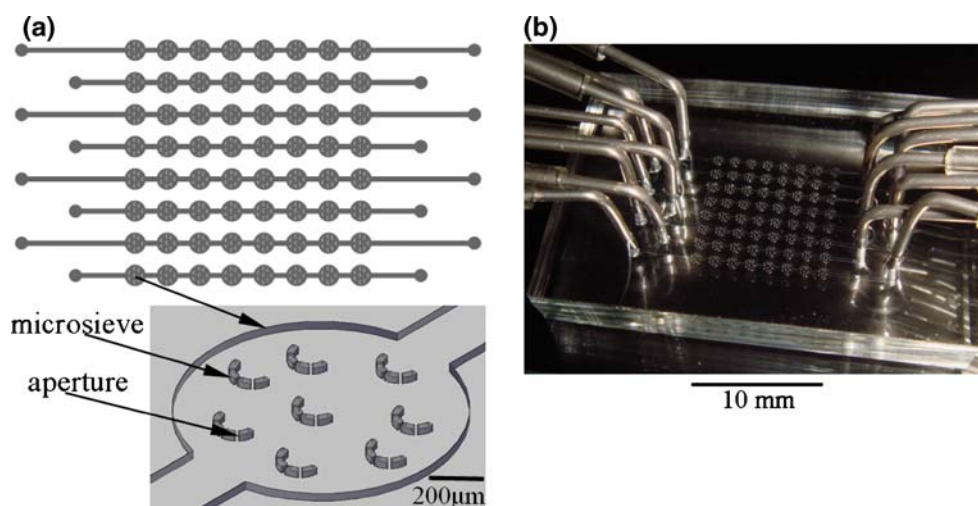
from Gibco Invitrogen Corp. All cell culture work was carried out in sterile tissue culture hoods and cell culture was carried out in a 5% CO_2 humidified incubator at 37°C . BALB/3T3 cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% calf serum. Master stocks of the BALB/3T3 cell lines, grown in T25 tissue culture flasks (Corning), were maintained by trypsinizing them at the point of confluence (0.25% in EDTA, Sigma) and passaging them at 1:5 subculture ratio. Cells grown in the microfluidic microchambers were trypsinized at the point of confluence and passaged at a $\sim 1:3$ subculture ratio, determined by fraction of cells retained in the sieves upon subsequent flushing.

2.3 Microfluidic simulations

To monitor cell trapping tendency in each micro-structured sieve in the chamber, one-way coupled Lagrangian particle simulations were carried out using recently developed program, which we refer to as 'Lab-Chip-Designer' (LCD). The LCD program mainly consists of three parts; importing of pre-computed flow field data, calculating of particle motion equations, and post-processing of cell trajectories (Wang et al. 2007).

Before importing flow field data into the LCD program, computational fluid dynamics (CFD) simulations were carried out using finite volume method-based commercial CFD tool (STAR-CD version 3.15a, CD-adapco). A SIMPLE (Semi-Implicit Method for Pressure Linked Equation) algorithm with tolerance of 1.0×10^{-5} was applied to solve the momentum and continuity equations. To emulate the solutions commonly used in microfluidic devices (i.e. buffers), an aqueous solution with a density 997.5 kg/m^3 was used as the model working fluid. Additionally, a flat velocity profile was applied at the initial microchamber inlet with a uniform flow rate of $1 \text{ } \mu\text{L/min}$.

Fig. 1 (A) Schematic of the 8×8 chamber microfluidic cell culture array platform. Each chamber contains eight micro-sieves for cell trapping. Each micro-sieve is semicircular with an interior diameter of $90 \text{ } \mu\text{m}$, $40 \text{ } \mu\text{m}$ (h), $20 \text{ } \mu\text{m}$ (w), with three apertures ($8 \text{ } \mu\text{m}$ width). (B) Image of the microfluidic device with fluid interconnects



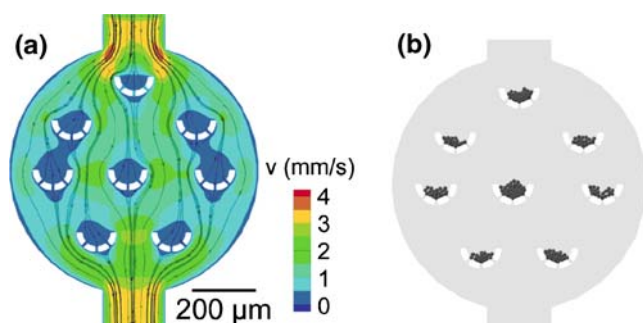


Fig. 2 (A) Theoretical flow velocity profile through single cell culture chamber with eight microsieves. Eight low flow velocity regions are formed within the chamber. (B) Simulation results for cell capture based on an input cell density of 10^6 cells mL^{-1} , a flow rate of $1 \mu\text{L min}^{-1}$ through the chamber, and a total flow time of 1.5 min

Pre-computed flow field data was used to evaluate forces acting on the spherical cell, including Stokes drag and pressure gradient forces, using a spatial interpolation method (Kim et al. 2008). An equation of motion for each individual cell was superimposed on these flow fields, taking into consideration the gravitational force, elastic spring force, and a diffusive force due to Brownian motion. The migration direction and distance for each cell was evaluated using Gaussian distribution under adaptively controlled time-step at every iteration (Zhao et al. 1997). Each cell was modeled as a $10 \mu\text{m}$ diameter sphere in aqueous solution seeded at the bottom of the channel randomly distributed across the inlet. To visualize dynamic cell distribution vs. time, data was exported to generate movie frames in Tecplot, a commercial CFD post-processing software package.

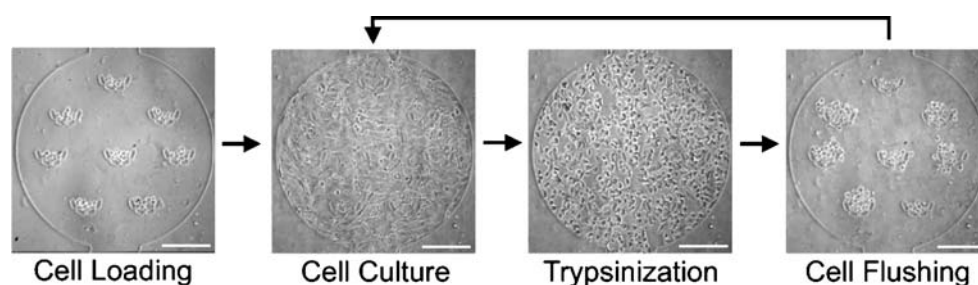
3 Results and discussion

A self-contained microfluidic cell culture array platform was designed to perform cell culture and iterative subculture. As depicted in Fig. 1, this cell culture platform consists of 8×8 culture chamber array. Each culture chamber is 0.8 mm in diameter and $40 \mu\text{m}$ in height. Eight microsieves are designed in each culture chamber for cell trapping. Each microsieve has three apertures, each with a width of $8 \mu\text{m}$.

Cell trapping was simulated as described in Materials and Methods for a cell culture chamber containing eight microsieves with a volumetric flow rate of $1 \mu\text{L/min}$. The simulation, coupling the fluid velocity profiles obtained through CFD for a given microchamber architecture (Fig. 2(a)) and Lagrangian tracking of single cells in the imposed flow field, generates a graphical model of the cell trapping process that correlates well with experimental results (Wang et al. 2007) (Fig. 2(b)). The fluid velocities in the regions surrounding the microsieves are substantially lower, as expected, with the apertures providing a reduced, but finite, flow of liquid through the sieves to draw in and trap the cells passing through the chambers. As cells are loaded into a multirow device, with each row containing a series of microchambers, only a small fraction of cells become trapped within the microsieves, resulting in nearly simultaneous filling of the upstream and downstream microchambers in a given row. In larger scale cell-trapping devices (24 parallel microchannels channels with 24 microchambers/row), we observed no statistical difference with respect to the number of cells trapped per sieve as a function of chamber position for a fixed media flow rate/cell concentration.

An adherent cell line (BALB/3T3) was used to demonstrate the capability of this microfluidic platform for cell culture and subculture. Before loading the 3T3 cells into the microfluidic cell culture platform, the fabricated microfluidic devices were autoclaved, rinsed with PBS, and degassed by driving trapped air through the walls of the gas-permeable device. The fluidic networks were precoated with 20 mg/mL gelatin (Sigma) for 1 hour to promote 3T3 cell attachment. Excess gelatin was removed by rinsing with PBS. A suspension of 3T3 cells ($\sim 10^6$ cells/mL) was injected into the device through the inlets at a flow rate of $1 \mu\text{L/min}$ per channel for 1.5 min. Cells not trapped in the microsieves were carried to the device outlet in the flowing medium. Cell distribution in this microfluidic platform is quite uniform with an average of 85 ± 6.3 cells per chamber (Fig. 3). After loading, the cells were allowed to settle under static condition in an incubator ($37^\circ\text{C}/5\% \text{ CO}_2$) for $\sim 6 \text{ h}$. The device was aseptically connected with a peristaltic pump at 6 h intervals to perfuse channels with fresh medium at $1 \mu\text{L/min}$ for 10 min to replenish critical metabolites and remove potentially toxic

Fig. 3 BALB/3T3 cell continuous culture process in the microfluidic cell culture array platform. Continuous cell culture shown as four steps; cell loading, cell culture, trypsinization, and cell flushing. Scale bar, $200 \mu\text{m}$



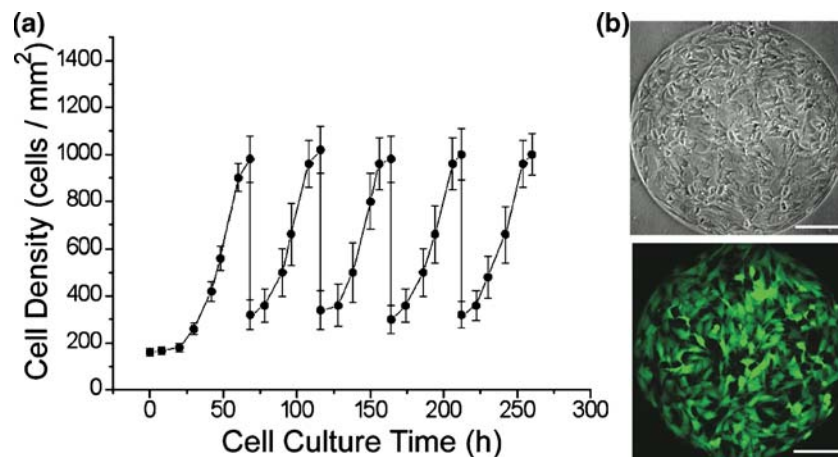


Fig. 4 (A) BALB/3T3 cell growth and subculture in the microfluidic cell culture array platform. Normal cell growth kinetics was observed over five cell subculture cycles, and the lag phase of 2–5 cycles was shorter than the first one due to a lower initial cell seeding density. (B)

Cell culture viability after five passages; Phase contrast image of a representative chamber (top) and the identical chamber/cells stained with LIVE/DEAD viability stain (bottom). Scale bar, 200 μ m

waste. After cells reached confluence (~ 500 cells/chamber) at ~ 65 – 70 h for the initial device seeding, trypsin (0.25% in EDTA, Sigma) was delivered into the microchambers at $1 \mu\text{L}/\text{min}$ for 5 min, followed by a static incubation for 20 min at 37°C . To remove the trypsinized cells, fresh medium was injected into the microchambers, flushing the majority of the cells out of the devices. Given the high surface-to-volume ratio of the microchambers, the flushed cells were quite concentrated ($\sim 10^7/\text{mL}$). The small fraction of cells retained in each microchambers (160 ± 29.5 cells), trapped by the microsieves, function to seed the culture for subsequent subculture. For each subculture cycle, the cells trapped after the flushing step were allowed to settle under static condition, then perfused with fresh medium until confluent again. Although the deviation is larger than that in the first cell loading cycle due to some cell aggregation, no decrease in cell viability was observed when the devices were used for multiple culture cycles.

The cell growth in culture chamber was monitored for five cycles (Fig. 4(a)). A lag phase of about one day was observed after cells loading, followed by an exponential growth phase. After the confluent layer formed, cells were trypsinized and re-trapped in the culture chamber. The lag phase in the following cycles was found to be shorter when seeding density was higher (average 160 cells per chamber) in comparison to the initial, lower density seeding. Cells spreading and maintenance of morphology in the microfluidic cell culture device was similar to that observed on tissue culture plastic, and cell viability was over than 99% after five subculture cycles measured with a LIVE/DEAD viability stain (Molecular Probes) (Fig. 4(b)). 3T3 cells cultured in the microfluidic devices and plastic flasks have approximately the same doubling time (18–20 h).

The channels in this platform are individually addressable, so multiple cell types can be seeded and cultured in the same time. In addition to T3 cells, we also successfully cultured

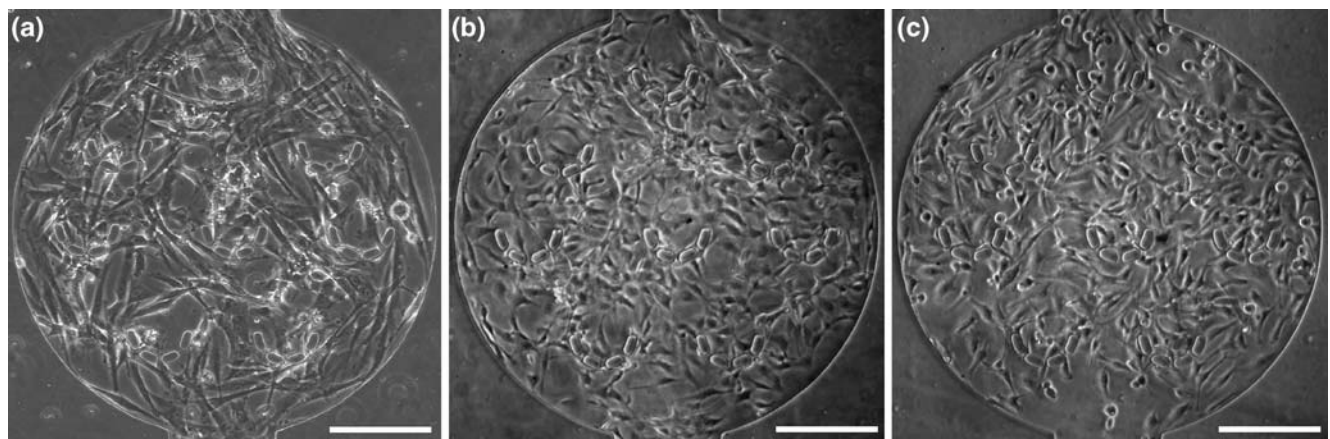


Fig. 5 Morphological observation of the cells cultured in the chambers, (A) mesenchymal stem cells, (B) HeLa cells, and (C) bovine endothelial cells. Scale bar, 200 μ m

HeLa, primary bovine endothelial cells and mesenchymal stem cell from human umbilical cord in separate rows of the devices in parallel, with comparable culture dynamics and viability/ culture cycle (cells morphology in chambers shown in Fig. 5), demonstrating the general utility of the platform. The scalable design of this microfluidic cell culture platform is suitable to enlarge into a high-density cell culture device to provide a sustained source of cells (multiple passages) for downstream applications, such as biochemical assays, and its flexible architecture can easily be adapted to directly interface with existing microfluidic modules designed for screening single (Carlo et al. 2006; Wheeler et al. 2003; Yang et al. 2002; Peng and Li 2004; Wu et al. 2004; Li et al. 2004) or small populations of cells (Press et al. 1992; Wheeler et al. 2003; Yang et al. 2002; Peng and Li 2004; Wolf et al. 2007; Shackman et al. 2005).

4 Conclusion

Conventional cell culture is a labor-intensive and time-consuming work, only can be performed by highly trained cell culture people with specific facility. The present microfluidic cell culture array platform has potential to develop into a fully automated cell culture system integrated with temperature control, fluidic control, micropumps. The self-contained microfluidic cell culture array platform will benefit many areas of cell-based research.

Acknowledgements This work was supported by an 863 project from Chinese Ministry of Science and Technology and a Korean Research Foundation Grant (KRF-2006-D00019).

References

- F.K. Balagaddé, L. You, C.L. Hansen, F.H. Arnold, S.R. Quake, Long-Term Monitoring of Bacteria Undergoing Programmed Population Control in a Microchemostat. *Science*. **309**, 137–140 (2005)
- D. Carlo, D.N. Aghdam, L.P. Lee, Single-cell enzyme concentrations, kinetics, and inhibition analysis using high-density hydrodynamic cell isolation arrays. *Anal. Chem.* **78**, 4925–4930 (2006)
- R. Gomez-Sjoberg, A.A. Leyrat, D.M. Pirone, C.S. Chen, S.R. Quake, Versatile, Fully Automated, Microfluidic Cell Culture System. *Anal. Chem.* **79**, 8557–63 (2007)
- H. Hufnagel, A. Huebner, C. Gülch, K. Güse, C. Abell, F. Hollfelder, An integrated cell culture lab on a chip: modular microdevices for cultivation of mammalian cells and delivery into microfluidic microdroplets. *Lab. Chip.* **9**, 1576–1582 (2009)
- C.W. Hung, G.B. Lee, A microfluidic system for automatic cell culture. *J. Micromech. Microeng.* **17**, 1266–1274 (2007)
- P.J. Hung, P.J. Lee, P. Sabounchi, R. Lin, L.P. Lee, Continuous perfusion microfluidic cell culture array for high-throughput cell-based assays. *Biotechnol. Bioeng.* **89**, 1–8 (2005)
- N.L. Jeon, H. Baskaran, S.K. Dertinger, G.M. Whitesides, L.V. Water, M. Toner, Neutrophil chemotaxis in linear and complex gradients of interleukin-8 formed in a microfabricated device. *Nat. Biotechnol.* **20**, 826–830 (2002)
- M.C. Kim, Z.H. Wang, R.H.W. Lam, T. Thorsen, Building a better cell trap: Applying Lagrangian modeling to the design of microfluidic devices for cell biology. *J. Appl. Phys.* **103**, 044701 (2008)
- K.R. King, S. Wang, D. Irimia, A. Jayaraman, M. Toner, M.L. Yarmush, A high-throughput microfluidic real-time gene expression living cell array. *Lab. Chip.* **7**, 77–85 (2007)
- P.C.H. Li, L. Camprieu, J. Cai, M. Sangar, Transport, retention and fluorescent measurement of single biological cells studied in microfluidic chips. *Lab. Chip.* **4**, 174–180 (2004)
- X.Y. Peng, P.H. Li, A three-dimensional flow control concept for single-cell experiments on a microchip. 1. Cell selection, cell retention, cell culture, cell balancing, and cell scanning. *Anal. Chem.* **76**, 5273–5281 (2004)
- S. Petronis, M. Stangegaard, C.B. Christensen, M. Dufva, Transparent polymeric cell culture chip with integrated temperature control and uniform media perfusion. **40**, 368–376 (2006)
- W.H. Press, S.A. Teukolsky, W.T. Vetterling, B.P. Flannery, *Numerical Recipes in C* (Cambridge University Press, Cambridge, UK, 1992)
- J.G. Shackman, G.M. Dahlgren, J.L. Peters, R.T. Kennedy, Perfusion and chemical monitoring of living cells on a microfluidic chip. *Lab. Chip.* **5**, 56–63 (2005)
- M. Stangegaard, S. Petronis, A.M. Jørgensen, C.B.V. Christensen, M. Dufva, A biocompatible micro cell culture chamber (μ CCC) for the culturing and online monitoring of eukaryote cells. **6**, 1045–1051 (2006)
- A. Tourovskaia, X. Figueroa-Masot, A. Folch, Differentiation-on-a-chip: A microfluidic platform for long-term cell culture studies. *Lab. Chip.* **5**, 14–19 (2005)
- Z. Wang, M.C. Kim, M. Marquez, T. Thorsen, High-Density Microfluidic Arrays for Cell Cytotoxicity Analysis. *Lab. Chip.* **7**, 740–745 (2007)
- A.R. Wheeler, W.R. Thordset, R.J. Whelan, A.M. Leach, R.N. Zare, Y.H. Liao, K. Farrell, I.D. Manger, A. Daridon, Microfluidic device for single-cell analysis. *Anal. Chem.* **75**, 3581–3586 (2003)
- M. Wolf, M. Zimmerman, E. Delamarche, P. Hunziker, Screening cell surface receptors using micromosaic immunoassays. *Biomed. Microdevices* **9**, 135–141 (2007)
- H.K. Wu, A. Wheeler, R.N. Zare, Chemical cytometry on a picoliter-scale integrated microfluidic chip. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 12809–12813 (2004)
- M.S. Yang, C.W. Li, J. Yang, Cell docking and on-chip monitoring of cellular reactions with a controlled concentration gradient on a microfluidic device. *Anal. Chem.* **74**, 3991–4001 (2002)
- X.M. Zhao, Y.N. Xia, G.M. Whitesides, Soft lithographic methods for nano-fabrication. *J. Mater. Chem.* **7**, 1069–1074 (1997)