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Apoptosis goes on a chip: advances in the microfluidic analysis of programmed cell death

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Professor Jon Cooper's interests lie in the application of micro- and nanotechnology in the biomedical sciences. He currently holds the Wolfson Chair in Bioengineering in the School of Engineering at the University of Glasgow. His group has developed a range of technologies associated with LOC devices. He has also been closely involved in the commercialization of a number of these technologies associated with cell technologies and medical diagnostics.

Summary

Recent years have brought enormous progress in cell-based lab-on-a-chip technologies, allowing dynamic studies of cell death with an unprecedented accuracy. As interest in the microfabricated technologies for cell-based bioassays is rapidly gaining momentum, we highlight the most promising technologies that provide a new outlook for the rapid assessment of programmed and accidental cell death and are applicable in drug discovery, high-content drug screening, and personalized clinical diagnostics.

Curing and eradicating cancer have been the main motivations behind the explosion of cancer biology research over the last four decades, yielding >2 million papers published so far. The important discovery that the induction of programmed cell death in neoplastic cells is a critical event that defines tumor growth rate and its regression or response to anticancer therapy has provided a framework for rationally designed and targeted anticancer therapeutics[1]. These studies have had an enormous impact on the understanding of the clinical manifestation of tumorigenesis but have only recently shown that the conventional anticancer treatments can induce diverse cell death modes, including classical caspase-dependent apoptosis, caspase-independent cell death, necrosis, reproductive cell death (cell senescence), or cell death dependent on autophagy genes (Figure 1A)[2]. These noncanonical pathways reveal the previously unanticipated complexity of molecular cross talk within the cell death pathways, reflected in a diversity of phenotypes that are difficult to characterize using conventional technologies (Figure 1B)[3].

As recently reported, the new microfabricated chip-based analytical technologies that support dynamic, real-time, cell-based analysis are essential to uncover the intricacies of cell-to-cell variability and support accelerated anticancer drug discovery[4]. Microfluidic, or lab-on-a-chip (LOC), devices are now widely considered an enabling technology in cancer cell biology and experimental oncology[5] and are very often the platforms of choice to address the inherent complexity of cellular systems with massive experimental parallelization and 4D (multiparameter outputs plus time) analysis and sorting of single cells[6]. Low cell numbers, small reagent volumes, fast mass transfer kinetics, and rapid reaction times in the microfabricated environment provide the unique ability to monitor single-cell signalling dynamics, especially in rare subpopulations of cancer cells.

Why use miniaturized LOC systems?

Many limitations of conventional cell-based assays can be superseded by implementing recent technological advances in LOC platforms. Microfluidics aims to manipulate liquids at ultralow volumes in circuitries of small channels that have a cross-sectional area <1 mm^2 (Figure 2A)[7]. At the microscale, fluids exhibit unique characteristics dominated by viscous forces. The behavior of flow is characterized by Reynolds number ($Re = \rho UD/\mu$, where ρ is the density, U is the average velocity of the flow, D is the equivalent hydraulic diameter of the microchannel, and μ is the fluid viscosity). The Reynolds number expresses the ratio of inertial forces to viscous forces and is limited to <100 in most microfluidic systems. Under these circumstances, the fluid flow is strictly laminar with no inertia and the solute transport is dominated by local diffusion (Figure 2A)[8]. These properties greatly enable accurate delivery of drugs, both spatially and temporally (Figure 2B, C)[9]. Such features are not readily attainable using any conventional, macroscale technologies, and thus microfluidic solutions warrant a major “quantum leap” for studying cell biology.

LOC devices also greatly reduce equipment costs and increase sensitivity and throughput by implementing parallel processing. Because only low cell numbers and operational reagent volumes are required, dynamic cytomics on a single-cell level finally appears within

investigational reach[10]. Importantly, the transfer of traditional bioanalytical methods to a microfabricated format increases the resolution of bioanalysis and the sampling throughput and also allows the design of automated systems with innovative functionalities. The advent of microfluidic technologies is therefore one of the most innovative cytometric approaches to the analysis of rare cells and organelles[3, 11].

Single-cell, real-time analysis rationale

New therapeutic anticancer targets have to be thoroughly validated by functional live-cell assays[12] before being tested in animals or humans. Tumor cell death serves, in this respect, as a practical endpoint measurement in pharmacological profiling and compound library screening routines. Yet, despite the apparent simplicity of analyzing cancer cell survival after treatment, inadequate models often hamper the current assays. The commonly used approaches to study cancer cell death are based on endpoint assays, yielding information only at intermittent time points[13]. The very nature of cell death is, however, based on switching between slow decision-making processes and fast execution processes that occur with varying asynchrony in a cell population[14]. Drug-induced tumor cell death is thus a highly stochastic process.

This phenomenon has recently sparked a large interest in protocols for the real-time and dynamic quantification of tumor cell viability during drug screening routines. Many organic fluorescent probes such as propidium iodide, SYTOXGreen, SYTOXRed, and YO-PRO 1 can noninvasively track cell death events over extended periods of time without compromising cell viability or interfering with cytotoxic drug action[12–13, 15]. Furthermore, substituted cyanine SYTO probes and fluorescent Annexin V conjugates represent a promising class of markers that do not adversely affect normal cellular physiology[16]. Caspase activation has also recently been monitored on a chip using L-bisaspartic acid rhodamine 110 (D(2)R) probe in live Jurkat cells captured on the affinity surface of a microdevice coated with anti-CD95 monoclonal antibody[17]. Moreover, the Caliper Technologies Labchip (disposable cartridge chips etched in glass substrate; Caliper Life Sciences, Hopkinton, Mass.) platform has been used for high-throughput caspase activation assays using fluorogenic substrates in subnanoliter volumes of reagents[18]. Finally, most recently, Khanal and colleagues used MitoTracker Red to follow mitochondrial depolarization in primary porcine cardiomyocytes in a low-shear microfluidic culture chip[19].

As reported in the above examples, inert organic fluorescent probes do not interfere with cell viability when continuously present in cell culture medium, and their intracellular retention can be easily used for kinetic analysis of caspase-dependent apoptosis. The low-dose, continuous labeling procedure not only provides similar results to a standard endpoint staining protocol, but also allows a straightforward adaptation for LOC-based platforms with minimal protocol modifications[16, 20]. The overlapping spectra of organic fluorochromes profoundly hamper the design and implementation of multiplexed apoptotic assays[21], but development of semiconductor nanocrystals commonly called quantum dots has recently superseded this common problem of spectral mismatch[21]. Annexin V functionalized quantum dots and polarity-sensitive Annexin-based biosensors are feasible for real-time and multiparameter studies of apoptosis and can be used for high-throughput screening on microfluidic platforms[22]. Most importantly, the reduction of sample processing steps achieved with such a kinetic protocol is important for the preservation of fragile apoptotic cells[12]. These data provide a rationale for the development of a new generation of assays with vast applications in chip-based analytical technologies that allow for convenient tracking of differences in stochastic cell death sensitivity that often remain undetectable with conventional endpoint analyses[14, 23].

Even more fascinating are innovative label-less approaches. Tamaki et al. have recently developed a revolutionary scanning thermal lens microscope for detection of nonfluorescent biological substances with extremely high sensitivity and spatial resolution[24]. Thermal lens microscopy uses the photothermal effect of a nonradiative relaxation in a very small volume coupled with a rapid thermal conduction between a solid-liquid interface. This allows for kinetic monitoring of rapid intracellular events associated with apoptosis such as single-cell quantitative analysis of cytochrome c release from mitochondria during apoptosis[24].

Microflow cytometry

Progress in development of monoclonal antibodies as novel functional fluorescent probes has enabled flow cytometric studies on individual cells. During the last decade, these techniques have found significant applications in clinical diagnostics and drug discovery. An advantage of flow cytometry (FCM) is rapid analysis that can reach throughputs of tens of thousands cells per second (many times faster than achieved in a microfluidic format). However, the common drawbacks of conventional FCM are its complexity, limited portability, cost, and requirement for highly trained service personnel[3]. Also, each flow cytometric analysis requires a considerable number of cells (typically $>10^4$ cells mL^{-1}), and processing of the samples prior to analysis is time consuming, often involving several centrifugation steps that subject cells to additional stress[3].

Microfluidics offers an innovative alternative to FCM, known as microflow cytometry (μFCM)[25]. Microfluidic flow cytometers require a greatly reduced (even 20-fold) number of cells when compared with conventional FCM, providing a remarkable advantage for studies of primary cancer cells. The pressure-driven flow in these devices maintains physiological parameters of cellular microenvironments. The flow is usually controlled by step motor driven syringe pumps, positive air pressure applied to input reservoirs, or vacuum applied to output reservoirs and is much faster than electroosmotic flow, with particle velocities reaching up to several millimeters per second[25].

In contrast to conventional FCM, in which the transfer rate through the flow chamber can be as high as 10^4–10^5 cells s^{-1}, most microfluidic planar chips maintain much lower transfer rates of 10–300 cells s^{-1}.[25b] This is beneficial for preservation of live cells because they are not adversely affected by the extensive pressure and associated shear stresses. Moreover, the reduced transfer rates can be effectively compensated for by simultaneous analysis of multiple parallel streams of cells on each chip[26].

Takeda and colleagues have made significant advances in planar μFCM by introducing the first commercially available, automated chip-based cytometric system with two way hydrodynamic sheath focusing and multilaser excitation with up to four color detection (the Fishman-R microfluidic flow cytometer developed by On Chip Biotechnologies Ltd, Tokyo, Japan; Figure 3A)[27]. Cells on planar microfluidic chips are hydrodynamically focused into a single file and then interrogated one by one by independent laser beams (Figure 3B, C). The sensitivity of this portable microfluidic cytometer falls within the already stringent specifications of conventional flow cytometers (FITC < 600 MESF, PE < 200 MESF; MESF = Molecules of Equivalent Soluble Fluorophore). Moreover, this is also the first μFCM that includes true Forward and Side Light Scatter detection (Figure 3A, C). The latter parameter, which is particularly important for the estimation of cellular complexity (especially granularity), utilizes SLER (Side scattered Light detection using Edge Reflection of chip) technology[27]. Another useful innovation of the chip is the design of postanalysis cell collection, a feature not readily achievable in any conventional system (Figure 3B). This capability may be useful for the preservation of rare cell subpopulations, such as primary cancer stem cells, allowing functional and molecular studies to be performed after

cytometric analysis[27]. The enclosed nature of microcytometers also makes them particularly suitable for analysis of highly infectious samples, such as virally-infected primary cells, or for testing virus-based vectors for gene delivery, as well as for analyzing samples that may contain genotoxic agents.

Microcytometers are sometimes criticized for having much lower detection sensitivities than conventional analyzers, particularly for FITC fluorescence detection (>2 million MESF, as opposed to <500 MESF, respectively)[28]. Recent reports have proven, however, that the sensitivity of μFCM is adequate to detect subtle changes in fluorescence intensity during microcytometric analysis of apoptosis. Using planar and vacuum driven Caliper LabChips, which can analyze six low volume samples simultaneously, rapid two-color quantification of apoptosis on small cancer cell samples is possible without special laser alignment and operator engagement[28]. The frequency of cancer cells detected with this setup in viable, apoptotic, and necrotic/late apoptotic stages does not differ more than 10% as compared to the reference conventional flow cytometer. Although chip to chip variation significantly exceeded variations observed using independent conventional cytometers, the excellent correlation between the two technologically dissimilar platforms provides further support for the development of automated microfluidic cytometers[28].

The progress in μFCM leverages many advances in microfluidic technology for single-cell analysis. The ultimate goal is, however, to produce user-friendly, portable devices capable of multiparameter fluorescent interrogation of single cells. Low power consumption and portability of μFCM can support the development of mobile systems ready for deployment in resource poor areas or disaster zones[27].

Ultrahigh throughput microflow cell sorting

The accurate separation of defined cell subpopulations is of paramount importance for cancer cell biology, clinical diagnostics, and patient-tailored therapies. Separation and subsequent analysis of living and dying cells, and particularly cells at different stages of apoptosis, constitutes a vital part of discovering the mechanisms regulating cell death and/or facilitating cell survival. Dynamic labeling and subsequent SYTO-based cell sorting of intact apoptotic cells has recently provided an innovative approach to fluorescently track progression of apoptotic cascades at the single-cell level[28]. Conventional FCM still remains the technology of choice, especially for rapid quantification and cell separation using high-speed, fluorescently-activated cell sorting[29]. However, there is an increasing interest in and demand for cost-effective and sterile cell sorting systems that will supplement FCM, especially for high-throughput cell separation during drug screening routines and clinical grade cell sorting[30].

A recent innovation in μFCM is development of the integrated Gigasort Clinical Grade Cell Sorter (CytonomeST LLC, Boston, Mass.), which is the fastest fully sterile optical cell sorter ever produced (>7× the throughput of any conventional droplet-based cell instrument). Disposable chip sorting cartridges enable gentle, clinical-grade, sterile sorting in a fully enclosed envelope, which has not been possible with other solid or droplet-based cell sorting methods (Figure 3D). The Gigasort's enclosed and disposable cartridges support staining protocols developed for conventional FCM with two excitation wavelengths (extinction and side scatter) and four fluorescence parameters from each cell. Within the device, microscopic particle switches (microsorters) operate at a rate of 2000 cell sorting operations per second (Figure 3E), an order of magnitude increase in rate observed over conventional systems. The increase in rate is obtained by minimizing the mass of fluid required for deflection, therefore maximizing switch acceleration, and by utilizing compressed gas pockets for actuator motion coupling and spring-like return characteristics. By using a

parallel array of up to 72 concurrently operating microfluidic sorter cores, the technology sorts at ultrahigh speeds of up to 500 million events per hour.

Microfluidic Live-Cell Arrays

A common drawback of flow cytometric analysis of cells and particles is that repetitive analysis of time resolved events is not possible because specimens are rapidly discarded[3]. Moreover, understanding of signaling pathways in the native cellular microenvironment in real time is limited because the collected fluorescence and/or electrical outputs are merely integrated as each cell passes the interrogation points. This design suffers from the loss of both subcellular information and identification of many morphometric features that can only be obtained by high-resolution cell imaging[3].

The complexity of cell death signaling and cancer heterogeneity at the single-cell level can only be dissected when the position of every cell is registered and maintained over extended periods of time (i.e., during the treatment and real-time multiparameter analysis)[16, 20, 23]. Kinetic analysis of single-cell responses can provide content rich data sets that aid the validation of selected endpoint events, guided by computational models. LOC devices provide here a considerable technological improvement for fabrication of parallelized and fully addressable live-cell microarrays[31].

Extended cell culture in enclosed microfluidic systems fabricated in biocompatible polymers is currently possible because evaporative water losses are negligible. As opposed to μFCM, microfluidic cell arrays provide continuous monitoring of individual, nonadherent cells protected from the stress associated with shear flow and sequential pharmacological stimulations while providing vast reagent and specimen savings[16, 20]. The rapid exchange of stimulants at the microscale also facilitates studies on real-time and spatiotemporal responses of cell behavior to a changing microenvironment, experiments not possible with any microflow cytometric technologies.

Hydrodynamic positioning of single cells in an array of mechanical traps can provide an elegant method for trapping and real-time analysis of single spatially arrayed cells (Figure 4A)[16, 20, 32]. Trapped cells experience shear stress forces that are significantly lower than adherent cells not shielded by the micromechanical sieves. Hydrodynamic forces and elastic collisions between cells located within the traps facilitate single-cell positioning and deflection of remaining cells away from the micromechanical trapping regions (Figure 4B)[16, 20].

Recently, we have applied live-cell microarrays to the kinetic analysis of programmed cell death in hematopoietic cancer cells and hematopoietic cancer stem cells (Figure 4C). We have for the first time exploited real-time fluorescence assays based on substituted cyanine SYTO probes, fluorescent Annexin V conjugates, and plasma permeability markers such as propidium iodide and SYTOX for multiparameter analysis of tumor cell death (Figure 4C, D) [16, 20]. Moreover, at the same time, we were able to track the cancer stem cell division symmetry, proliferation rate, and motility in response to the small molecule kinase inhibitors. Noninvasive tracking of intracellular events over extended time provided new understanding of the process of caspase-dependent apoptosis and alternative cell death modes.

In contrast, the serial observation of cells by FCM or even μFCM produces only population averages that cannot identify whether separate populations of cells exhibit different time courses[3, 23]. Therefore, microfluidic live-cell arrays are an emerging technique with substantial applications in high-throughput screening. They are poised to provide a new dimension to the on-chip studies of patient-derived normal and cancerous stem cells

otherwise difficult to perform using macroscale methods. They also can monitor in real time cell viability in high capacity cell culture bioreactors used in the biotechnology and pharmaceutical industries.

Bioelectrical cytometry

Dielectrophoresis (DEP), the induced motion of polarizable particles in nonhomogenous electric fields, is a versatile technique to characterize different cell lines in microfluidic systems[33]. The polarization of cells with respect to their surrounding medium depends on three factors: 1) the dielectric properties (conductivity and capacitance) of cells, 2) the dielectric properties of the medium, and 3) the frequency of the applied AC signal[34]. The overall dielectric properties of a cell in turn depend on the dielectric specifications of the cell's interior and plasma membrane. These specifications represent cells' intercellular reactions, surface morphological complexities, and membrane barrier functions and strongly respond to cell physiologic and pathologic changes[34].

The DEP response of cells is characterized by the Clausius-Mossotti factor, as later referenced in the text, $\mathrm{Re}[f_{CM}]$. If $\mathrm{Re}[f_{CM}] > 0$ the cells are pushed towards the regions of strong electric field formed at the tips of microelectrodes, and such a motion is called positive DEP (pDEP). Conversely, if $\mathrm{Re}[f_{CM}] < 0$ the cells are pushed away from the regions of strong electric field, and such a motion is called negative DEP (nDEP). Accordingly, at $\mathrm{Re}[f_{CM}] = 0$, the cell will experience a transition from positive to negative DEP or vice versa, and the associated frequency is called crossover frequency[35]. The dielectric properties of cells, including monocytic cells (U937), T-lymphocytes (Jurkat), T cell leukemia cells (Ind-2), blood mononuclear cells (PBMC), neuroblastoma cells (SH-SY5Y), and glioma cells (HTB)[36], can be determined by measuring their crossover frequency at different medium conductivities.

The dielectric properties of cells can also be determined using an electrorotation technique in which the cells are subjected to a rotating electric field produced by quadrupole microelectrodes (the sequential microelectrodes are energized with 0°, 90°, 180°, and 270° signals). The cell reaches a steady rotational speed after the induced rotational torque and the frictional torque arising from the medium viscosity reach a balance[37]. The dielectric properties of a cell can be determined by measuring its rotational speed at different frequencies, as reported for different cell lines, including human breast cancer cells MDA231, T-lymphocytes, and erythrocytes[37].

Dielectrophoretic Cell Arrays

DEP is capable of immobilizing the target cells at the predetermined locations of the microfluidic channels. The cells can be trapped in large or small clusters or individually. We have recently shown the performance of a DEP system that takes advantage of curved microelectrodes (Figure 5A). The microelectrode arrays generate weak electrothermal vortices, acting as conveyor belts and transferring the cells from all parts of the microchamber towards the microelectrodes, where they are trapped under the attractive DEP force.

This system rapidly immobilized human histiocytic leukemia U937 cells at the delta-shaped region enclosed between the opposite microelectrodes (Figure 5B and C)[15]. This proved useful for accelerated real-time cytotoxicity assays on cells immobilized in DEP fields. We showed that dynamic assessment of drug induced cytotoxicity can be reliably achieved on DEP chips with subsequent high-resolution fluorescent imaging (Figure 5D). The DEP-based arrays significantly reduced the complexity of conventional protocols and enabled time-resolved studies on immobilized hematopoietic (nonadherent) cells[15]. The latter feature

is of particular advantage for determining the kinetics of pharmaceutical efficacy in these nonadherent cell lines, allowing for sequential pharmacological stimulations and real-time analysis of cellular physiology with reduced amounts of reagents and sample (Figure 5E). Most importantly, such DEP technologies allow the simultaneous immobilization of small cell clusters rather than isolated single cells[15], which permits short-range cell-to-cell communications. This ability is of particular importance in studying the outcome of patient derived cancer cells that have been exposed to therapeutic drugs because these cells are often rare and difficult to collect, purify, and immobilize[15].

Alternatively, small clusters of 1–5 cells can be trapped using cage microelectrodes composed of two metal rings or extruded microelectrodes composed of four cylindrical posts in a trapezoidal arrangement and excited in a quadrupolar fashion[38]. Both configurations create an electric field cage in their centers where the cells are levitated under the repulsive DEP force and permit the stable immobilization of flowing cells even in the presence of relatively high velocities[38b]. The trapped cells are not exposed to strong electric fields formed at the edges of microelectrodes and do not experience large transmembrane potential changes. Moreover, by controlling individually addressable electrodes, selected single cells or small clusters of cells can be released from the trapping region at any given time. The major advantage of these DEP-based arrays is that cells can be 1) transiently trapped, 2) stimulated with chemical compounds of interest, and 3) subsequently released for further processing[38a, 39]. Despite these advantages, these DEP arrays are very costly and complex to manufacture because they require fabrication of multiple electrodes units for every single-cell trap[15, 33].

Conclusions

Microfluidic LOC technologies provide straightforward and effective solutions for low-cost, high-throughput screening at a single-cell level. Development of these novel technologies capable of performing traditional tasks in a faster, more economical, and precise manner can benefit a number of areas including clinical diagnostics and future high-content screening routines[5]. Importantly, because LOC assays require only ultralow cell numbers, the ability to monitor single-cell dynamics of rare cell subpopulations such as stem cells or fine needle biopsy aspirates appears finally within investigational reach. For studies of cellular responses to novel targeted anticancer agents, the manipulation of laminar flows also allows the precise, rapid, and gentle delivery of reagents to cells of interest. This is particularly advantageous in deciphering kinetic cell responses to novel pharmacological compounds. Increasing numbers of reports provide innovative solutions that reach beyond simple proof-of-principle studies to applications of microfluidics in drug discovery and clinical diagnostics.

Recent progress in the field shows that novel LOC technologies such as microfluidic cell arrays will greatly transform anticancer drug discovery and clinical oncology in years to come. Microfluidic technologies will likely become the next evolutionary step for dynamic studies of cell death with an unprecedented accuracy. They will provide new validation of many systems biology models with large populations of patient-derived cells using simultaneous analyses in which the position of every cell is encoded and spatially maintained over extended periods of time. By combining diverse analytical capabilities, LOC technologies will become very practical tools for the high-content studies on tumor cell biology and anticancer drug discovery. A dramatic increase in throughput and automation of such devices could greatly support the emergence of systems oncology and truly personalized anticancer therapy.

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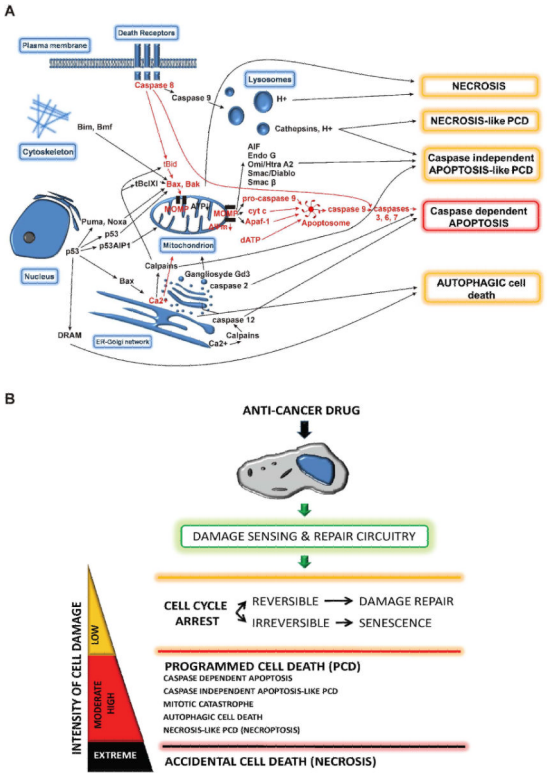

Figure 1.

The programs of cell death. A) Diagram of current concepts on the complexity of cell death modes. A plethora of molecular pathways are present in every cell and regulate cell death programs in response to both internal and external stimuli. PCD = programmed cell death. B) A cancer cell exposed to an anticancer drug can respond differently depending on the signal strength. Committing to cell death varies depending on the inducer type, cell type, and the “time window” in which the process of cell death is observed. Sublethal doses of the drug can initiate self-repair mechanisms or push a cell toward replicative senescence.

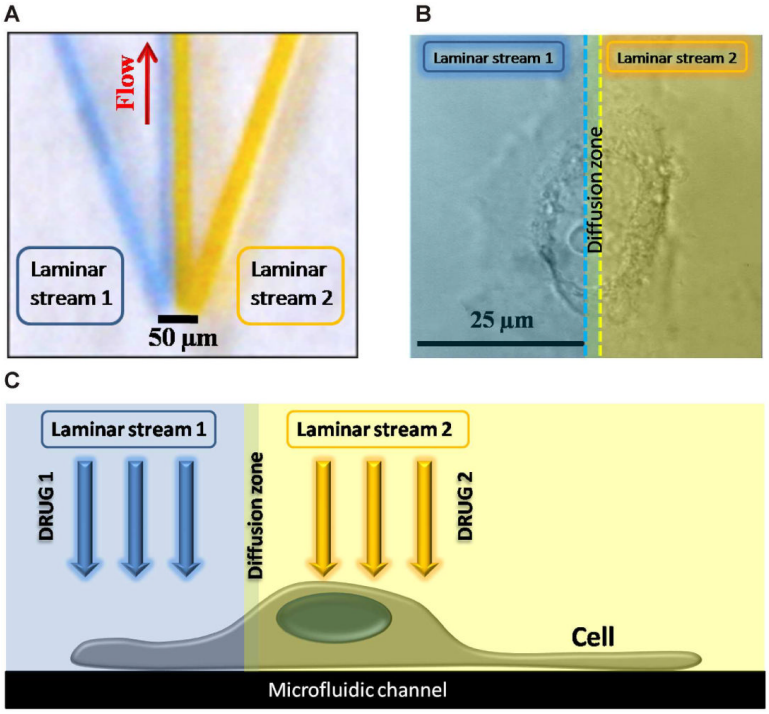

Figure 2.

Principles of microfluidics for cell biology. A) During fluid flow under low Reynolds numbers, solute transport is dominated only by a limited local diffusion. Generation of a multitude of fluidic domains that do not mix with each other is possible under these conditions. B) Example phase-contrast microphotograph showing a single human carcinoma HeLa cell stimulated using laminar flow under low Reynolds numbers. Blue (laminar stream 1) and yellow (laminar stream 2) denote drug delivery restricted to half-cell regions. The minimal local diffusion zone between the drugs is highlighted in the middle of the cell. C) Side view 3D model illustrating the situation in (B) and showing how laminar streams can be effectively used for spatiotemporal stimulation of distinctive cellular subdomains.

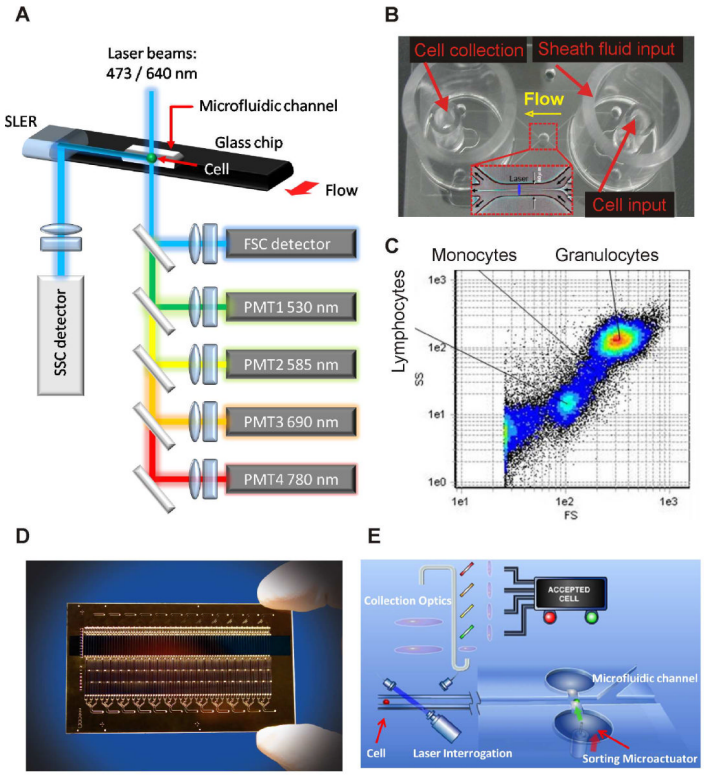

Figure 3.

Cell sorting with μFCM. A) Optical path of an advanced microfluidic flow cytometer (Fishman-R). FSC (Forward Light Scatter), SSC (Side Light Scatter), and four fluorescence detectors are used in combination with spatially separated solid state 473 nm and 640 nm lasers. Side scatter detection is performed using SLER (Side scattered Light detection using Edge Reflection of chip) technology. Reproduced from Ref 4a with permission of Elsevier. B) Disposable microfluidic cartridge used by the Fishman-R technology. The planar chip is designed to provide 2D hydrodynamic cell focusing of cells into single file. Image courtesy of Dr. Kazuo Takeda (On Chip Biotechnologies Co Ltd, Tokyo, Japan). Reproduced from Ref 4a with permission of Elsevier. C) Analysis of whole blood leukocytes using Fishman-R microflow cytometry technology. Image courtesy of Dr. Kazuo Takeda (On Chip Biotechnologies Co Ltd, Tokyo, Japan); D) Gigasort Clinical Grade Cell Sorter is a microfabricated glass chip measuring 2 × 3 inches and containing 72 parallel microfluidic switches (microsorters). E) Diagram of a single Gigasort microsorter.

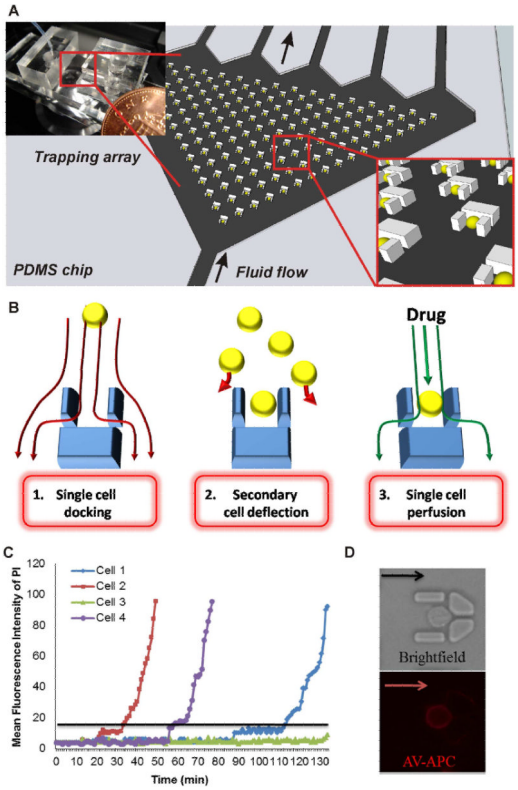

Figure 4.

Microfluidic live-cell arrays. A) A microfluidic chip with a triangular chamber contains a low-density cell positioning array. Bottom right: A magnified view of 3D model cells of the cell trapping region. Top left: A photograph of a device fabricated in a biocompatible elastomer, PDMS. B) Diagram of single-cell docking and microperfusion on a microfluidic cell array. The design allows the hydrodynamic positioning of single cells inside the micromechanical traps. Inside the traps, a low shear stress zone is characterized by a velocity field much lower than in the remaining area of the microchamber. C) Quantitative and kinetic analysis of programmed cell death at a single-cell level on a microfluidic cell array. Representative cells were selected and their integrated fluorescence (Mean Fluorescence Intensity) measured following incorporation of plasma permeability marker propidium iodide (PI). Single-cell level analysis was able to resolve stochastic responses to a kinase inhibitor. Reproduced from ref. 6 with permission from Springer. D) Dynamic analysis of apoptosis in cells immobilized in micromechanical traps on a chip. Human promyelocytic leukemia cells were perfused with pan-kinase inhibitor staurosporine in the presence of fluorescently conjugated Annexin V (AV-APC; red). Staining with AV-APC marks the externalization of phosphatidylserine residues characteristic of early apoptotic stages. Arrows indicate direction of laminar flow. Reproduced from ref. 6 with permission from Springer.

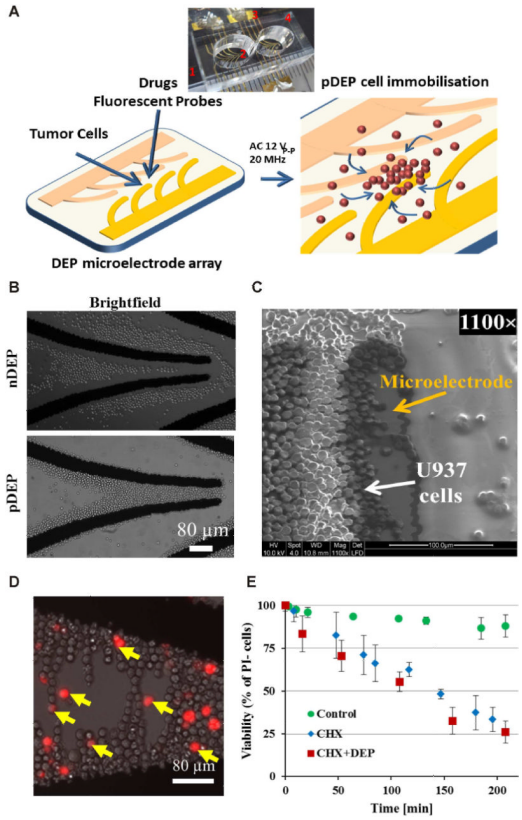

Figure 5.

Dielectrophoretic cell microarrays. A) Schematic showing the principles of cell immobilization in a spatially nonuniform electric field generated by curved microelectrodes. Top photograph: 1 = glass substrate; 2 = microfabricated chrome/gold electrode array; 3 = interconnecting pads providing AC signal actuation; and 4 = PMMA/PDMS microculture chamber. Reproduced from ref. 15. B) Human histiocytic leukemia U937 cells trapped in nDEP (upper panel) and pDEP (lower panel) fields on the microelectrode array. Reproduced from ref. 15. C) SEM micrographs of U937 cells immobilized in a spatially nonuniform electric field. D) Analysis of cytotoxicity in DEP-immobilized U937 cells under continuous challenge with the cytotoxic drug cycloheximide and dynamic labeling with a marker of plasma membrane permeability, propidium iodide. Dead cells are stained bright red with propidium iodide (yellow arrows). Data indicate that bioassays detecting cell viability can be readily adapted for novel DEP platforms with minimal protocol modifications. Reproduced from ref. 15. E) Real-time cytotoxicity analysis on DEP arrays can provide stratified cell survival curves that do not differ significantly from analysis using conventional methods. CHX = cycloheximide, protein synthesis inhibitor. Reproduced from ref. 15.