Chapter 1

MICROFLUIDIC DEVICES FOR CELLOMICS

A generaloverview of the field

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Abstract: A review of microfluidic devices for cellomics is presented. After a brief description of the historical background of Lab-on-Chip (LOC) devices, different areas are reviewed. Devices for cell sampling are presented, followed by cell trapping and cell sorting devices based upon mechanical and electrical principles. The next section describes devices for cell treatment: cell lysis, electroporation and cell fusion. Finally a number of microfluidic devices for cell analysis are reviewed, including cell transport and cultivation, electrical and mechanical characterization, and finally biochemical sensing. Most of these areas will be treated in depth in the next chapters of this book.

Key words: cells, microfluidic devices, Labs-on-chip

1. INTRODUCTION

In the past ten years there has been an increased interest in research on so-called Micro Total Analysis Systems (μTAS) or Labs-on-a-Chip (LOC) as illustrated by the rapid growth of the international μTAS conference, the appearance of an entirely new journal ["Lab-on-a-Chip"], a special section on this topic [μTAS in S&A B], and many articles appearing in related journals [Electrophoresis, Journal of Chromatography A, Analytical Chemistry] as well as several articles reviewing this topic in more or less detail [1-5]. Initially, there were two approaches followed in this field: one aiming at combining microsensors with fluidic components (pumps, flow sensors) into systems (*e.*g. ammonia/phosphate sensing) [6-7]; the other, which had a much greater impact, focused on miniaturization of analytical chemical methods, in particular separations, with after the first

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demonstration with amino acids [8] a lot of emphasis on genetic (DNA) analysis [9-11]. As genetic analysis has now become a more or less routine method, the new focus has been for some time, and still is, on using μTAS systems for protein analysis [2]. In addition, in the past few years, the interest in analysis of even more complex biological systems such as living cells with the use of microfabricated structures has attracted increased attention. Thus, the application of microfabrication techniques has really entered the life science field and has started to serve as a driving force for discovery in cell biology, neurobiology, pharmacology and tissue engineering. There are several reasons making microfluidic devices and systems interesting for cellomics:

- increased interest in biochemical experimentation/analysis of living single cells e.g. for studying effects of drugs, external stimuli on cell behavior etc.
- possibility of easy integration of all kinds of analytical standard operations into on microfluidic system
- several methods for manipulating large numbers of cells simultaneously can be used in microfluidic systems
- the size of cells fits very well with that of commonly used fluidic devices (10-100 um)
- micromechanical devices are very well capable of manipulating *single* objects with cellular dimensions.

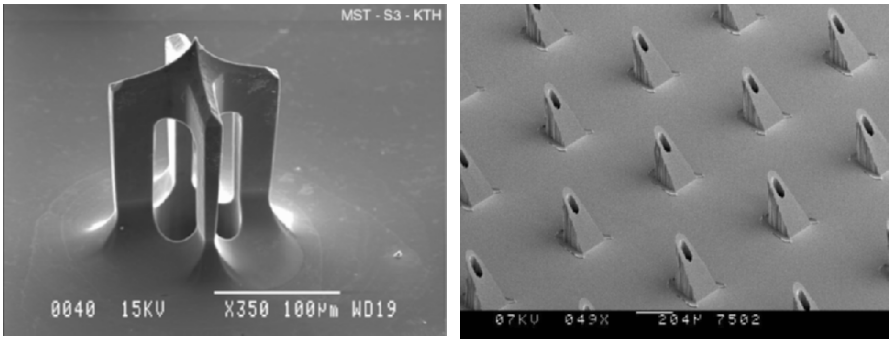
As the field of cellomics is expected to become a very important one, the motivation for writing this chapter is to provide an overview of what has been achieved and realized so far with microfluidic devices and systems for analysis of living cells.

2. CELL SAMPLING

Today diagnostic sampling in most cases requires extraction of blood through a hypodermic syringe needle, followed by analysis of blood components in a laboratory environment. During the last decades, it has become clear that the introduction of microfabricated devices offers exciting opportunities to advance the medical field such as minimally invasive procedures and portable devices. Several approaches to the micromachining of this type of device are known, and roughly these can be divided in in-plane and out-of-plane designs, the plane in this case being the surface of e.g. a silicon wafer. The in-plane version is the most convenient to fabricate with state-of-the-art planar technology [12-14], comprising surface micromachining and different techniques of silicon etching, and creates a good degree of flexibility with respect to needle design. However, the

density of needles that can be obtained is limited, and the strength of in-plane needles is often limited. A disadvantage is that their flat hollow tips tend to punch and therewith damage the skin, whilst the punched material may at least partially obstruct liquid flow through the needle. Stoeber *et al.* [15], who used directional Reactive Ion Etching (RIE) to define a narrow flow channel obtained very promising results through a silicon wafer and thin film protection of this channel followed by isotropic etching from the other side of the wafer to fabricate the needle. This method allowed the fabrication of robust needles with a flow channel off-center of the needle tip, which reduces the punching problem described above. However, the radius of the tips was relatively large and needs further improvement.

A totally different and very original approach was followed by Griss *et al.*, who fabricated out-of-plane microneedles with openings on the side, by which clogging due to punching is avoided [16], see figure 1 (a). Other micromachined hollow microneedles for extraction of blood have been presented [17-18], see figure 1 (b). Tests have shown that capillary forces draw the blood into the needle, reducing the need for external pumping means. Future research will focus on the connection of a complete microsystem for blood analysis to the needle chip, and it is clear that the combination of microfluidic devices, analysis system and microneedle can be a very powerful one.



(a) (b)

Figure 1-1. Examples of microneedles for cell sampling (Panel (a) reprinted from Griss, P. and G. Stemme (2002) Novel, side opened out-of-plane microneedles for microfluidic transdermal interfacing, MEMS 2002, 467; paner (b) reprinted form Gardeniers, J., et al., (2002) Silicon micromachined hollow microneedles for transdermal liquid transfer, MEMS, 141-144, with permissions from © 2002 IEEE).

3. CELL TRAPPING AND SORTING

The current methods commonly used in biological laboratories for manipulation, concentration, and separation of bioparticles include optical tweezers, fluorescence or magnetic activated cell sorting, centrifugation, filtration and electric field-based manipulations and separations. In the following sections a few miniaturized concepts for trapping and sorting cells will be described.

3.1 Mechanical Trapping and Sorting

Trapping biological particles mechanically on a microchip poses challenges because of the complex physical properties of biological particles. White blood cells, for example, are extremely sticky while red blood cells are rather non-adhesive. Microfabricated mechanical filters have been described for trapping different cell types from blood [19-21]. These filters were made of arrays of rectangular, parallel channels on chip of a width and height that would not allow particles larger than the channels to enter the channel network along the axis parallel to the chip surface. Carlson *et al.* [19] and Bakajin *et al.* [20] used hydrodynamic forces to move the blood through a lattice of channels. While the red blood cells readily penetrate and pass through the lattice, the white cells are greatly retarded and eventually adhere to the surface. The white blood cells self-fractionate into the different types of white cells. Andersson *et al.* [22], used deep reactive ion etching (DRIE) to fabricate a confined volume surrounded by vertical silicon bars. This microreactor volume is well suited to trap beads, and is currently investigated for trapping of cells. Wilding *et al.* used a microchip containing a series of 3.5 μm feature-sized weir-type filters formed by an etched dam spanning a flow chamber to isolate white blood cells from whole blood. Genomic DNA targets can be directly amplified using PCR on the cells captured on the filters [21].

A cell filter fabricated in quartz consisting of a network of intersecting 1.5 x 10 μm channels was shown by He *et al.* [23]. When placed at the bottom of reservoirs with a side-exit this channel network behaved as a lateral percolation filter composed of an array of cube-like structures one layer deep. This filter showed to be efficient in trapping animal cells and *E. coli*.

A biomimetic method for cell separation based on adhesive rolling and transient tethering has been demonstrated in microstructured fluidic channels by Chang *et al.* [24]. Using E-selectin-ligand adhesions, capture and several hundred-fold enrichment of HL-60 cells on channel surfaces under continuous sample flow was achieved.

A totally different approach to trap cells may be obtained by using micropipettes. These devices have been successfully used by Rusu *et al.* to aspire beads and draw them out of an optical trap, and may be interesting tools to manipulate individual cells [25].

3.2 Electric Trapping and Sorting

Among the many manipulation techniques, the electric field-based approach is well suited for miniaturization because of relative ease of microscale generation and structuring of an electric field on microchips. Furthermore, electrically driven microchips provide the advantages of speed, flexibility, controllability, and ease of application to automation. Depending on the nature of bioparticles to be manipulated, different types of electric fields can be applied: 1) a DC field for electrophoresis of charged particles, 2) a nonuniform AC field for dielectrophoresis (DEP) of polarizable (charged or neutral) particles, 3) the combined AC and DC fields for manipulating charged and neutral particles. On the microchip scale electrophoresis has been used in conjunction with electro-osmosis for electrokinetic transportation and separation of molecules and cells in microchannels [26-30]. Because most biological cells have similar electrophoretic mobilities, electrophoresis for manipulation of cells has limited applications and is almost exclusively used for pumping (electroosmotic flow, EOF).

On the other hand, DEP has been successfully applied on microchip scales to manipulate and separate a variety of biological cells including bacteria, yeast and mammalian cells [31-40]. For example, DEP enrichment in a flow cell of microliter volumes has been shown for concentrating *E.coli* (20 times) from a diluted sample and peripheral blood mononuclear cells (28-fold enrichment) from diluted whole blood [40]. A 30-fold enrichment of white blood cells from diluted whole blood has been achieved [40]. Particle concentration and switching have been shown by Fiedler *et al.* [31] for linear flow velocities up to about 10 mm/s. Application of DEP for separating and transporting cells and bioparticles on microfabricated arrays has been described by Xu *et al.* [37]. A multiple-force chip comprising electromagnetic elements and DEP electrodes for integrated cell and molecule manipulation was shown by Xu *et al.* [36]. White blood cells were separated by DEP, lysed and the released mRNA was bound to labeled magnetic beads, which were retained whilst removing the other molecules. The beads were then released for off-chip collection.

Electrodeless dielectrophoretic traps have been fabricated in an insulation substrate composed of geometrical constrictions [41]. The constriction is used to squeeze the electric field in a conducting solution, thereby creating a

high field gradient with a local maximum. Trapping of *E. coli* and its separation from blood cells in various salt concentrations have been demonstrated.

3.3 Flow Cytometry on Chip

In flow cytometry the particle jet is produced by hydrodynamic focusing in a sheath fluid. Optical signals are collected as the particles pass the detector. To sort, the jet is broken into droplets by a nozzle, and droplets containing chosen particles are electrostatically deflected. A throughput of the order of 10^4 cells/s is common with available machines. Conventional fluorescence activated cell sorters (FACS) suffer from the discrepancy between tool and object size. This mismatch hinders their integration with miniaturized, high-performance analytical systems. Only a few examples of cell and particle transport or sorting on microfabricated devices have appeared where hydrodynamic [42-43] electrokinetic [44], electroosmotic [29-30] and DEP [31, 39, 45] forces have been presented.

Telleman et al. has shown magnetic and fluorescent activated sorting using laminar flow switching in microfluidic devices [42]. The magnetic particles sheathed with two buffer streams were separated from non-magnetic particles by deflection in a magnetic field gradient. A photomultiplier tube was used to detect the fluorescently labeled particles. The PMT switches a valve on one of the outlets of the sorter microstructure and selects the particle by forcing it to the collecting outlet.

Another microfluidic device for cytometry of fluorescently labeled E.coli samples was described by McClain *et al.* [44]. The channels of this device were coated to reduce cell adhesion, and consequently, the focusing was performed electrophoretically without electroosmotic flow. The cells were continuously transported past the detection window with throughputs of 30-80 Hz. Voldman et al. has developed a microfabricated device for use in parallel luminescent single cell assays that can sort populations of cells upon the basis of dynamic functional responses to stimuli [39]. This device is composed of a regular array of noncontact single cell traps, see figure 2. These traps use DEP to confine cells and hold them against disrupting fluid flows. Situating an array of these traps in a microchannel it was shown that cells could be loaded, optically observed and sorted based on their dynamic fluorescent response to a stimulus. In contrast to the approach used by Becker *et al.* [34] single cells can be manipulated in high conductivity buffers through the dielectric properties of the cells.

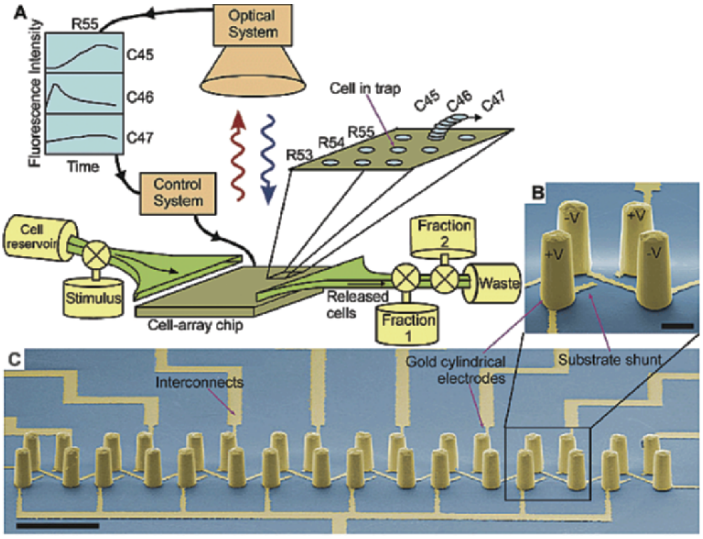


Figure 1-2. A schematic and pseudocolored scanning electron micrograph of the microfabricated. dynamic array cytometer (Reprinted from [39], copyrighth 2002, American Chemical Society).

Fu *et al.* [29] has constructed a microfabricated FACS device and demonstrated its effectiveness for sorting of microbeads and bacterial cells using electrokinetic flow. The disposable sorting device is fabricated using soft lithography, which enables the design of inexpensive and flexible miniaturized fluidic devices. The throughput of the device is about 20 cells/s. However, this electrokinetic device suffers from some drawbacks as all the electrokinetically actuated microfluidic devices, such as buffer incompatibilities and frequent change of voltage settings due to ion depletion, pressure imbalance, and evaporation. Fu *et al.* [46] has also presented a cell sorter, which has switching valves, dampers and peristaltic pumps. This sorter is also fabricated using soft lithography and it has improved throughput, buffer compatibility, automation and cell viability.

For the first time, Gradl *et al.* [47] and Muller *et al.* [48] introduced a novel microdevice for high contend cell analysis and sorting, see figure 3. This progress was based on previous by Muller *et al.* [45] and Fiedler *et al.* [31]. In this device, suspended single cells are freely floating in microchannels along a focal plane defined by the electromagnetic field which is generated by a particular 3D electrode configuration. This device also allows the stable entrapment of single cells in dielectric field cages against an applied flow of the medium. In these cages, the cells can be evaluated and analyzed through their fluorescent properties using spectroscopic and microscopic means. Subsequently, the cells are transported into a second sorting channel that gives access to the cells for

their single cell cloning under sterile conditions. In addition, Gradl *et al.* [47] showed that the combination of the 3D field cages and high resolution fluorescence allows the loading of cells with an additional agent (Calcein).

An interesting approach was presented by Gawad *et al.* [49] who used impedance spectroscopy for cell sorting. Using the real part of the impedance at two different frequencies he was able to effectively distinguish erythrocytes from ghost cells with a typical transit time in the order of 1 ms. A new type of coulter counter has been presented by Nieuwenhuis *et al.* [50] where the aperture is defined by a flow of non-conductive liquid that partially surrounds the sample liquid; changing the ratio of the flow rates of the two liquids allows adapting the diameter of the coulter aperture.

A microcytometry system that monitors leukocyte populations to assess human pathogen exposure is being jointly developed by Micronics and Honeywell [51]. The system contains both an instrument and a disposable card that contains complex microfluidic circuits for blood sample acquisition, reagent storage, erythrocyte lysis, cytometry and waste storage.

A miniaturized semiconductor-based laser-induced fluorescence detection system has been integrated onto a miniature prototype flow cytometry device by Kruger *et al*. [52]. Micro-optics, leaky waveguide coupling and solid-state detection have been combined with microfluidic technology to enable on-chip detection.

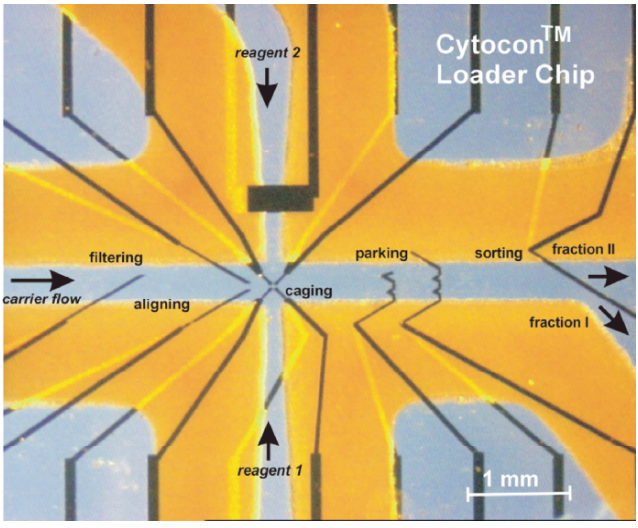


Figure 1-3. A cytocon™-Loader chip developed by Evotec technologies (Germany) for reagent application and sorting. The microchip consists of cross channels and two sorting channels. Microelectrodes form various elements for dielectrophoresis; funnel for alignment, zigzag for parking ablenkelement, straight electrodes as switch and deflector (www.evotec-technologies.com).

4. CELL TREATMENT

A critical requirement for achieving a Lab-on-Chip for the analysis of cells and their constituents is to integrate the cell treatment steps on chip. In the sections below some of examples of the development of cell lysis, genetransfection and cell fusion devices are presented.

4.1 Cell Lysis

Typical laboratory protocols for off-chip lysis steps include the use of enzymes (lysozyme), chemical lytic agents (detergents), and mechanical forces (sonication, bead milling). However, many such lysis techniques are not amenable to implementation in a microfluidic format. The ability to integrate the lysis of cells with the analysis of their contents would greatly increase the power and portability of many microfluidic devices.

Several research groups have developed microfluidic cell lysis devices. For example, an integrated monolithic microchip device was fabricated that used electrokinetic fluid actuation and thermal cycling to accomplish lysis of *E.coli* and PCR amplification of DNA [53]. In a similar electrokinetic device, the controlled manipulation of canine erythrocytes throughout a channel network and dark-field images of SDS lysis of the cells at a T-junction were demonstrated [30]. Other groups have reported the use of minisonicator devices in conjunction with microfluidics and glass beads for the lysis of spores [54-55]. Bacillus spores were successfully disrupted and ready for PCR in only 30 seconds. The microsonicator device significantly improved PCR analysis of the spores. In a different approach, a silicon channel was fabricated with microelectrode pairs along the walls to deliver an electric field to irreversibly electroporate several different cell types [56]. A voltage of 10 V was applied across gaps of several micrometers to achieve electric fields on the order of 1 to 10 kV/cm.

A microfluidic system integrating the continuous lysis of bacterial cells and the fractionation/detection of a large intracellular protein has been demonstrated by Schilling *et al.* [57]. This system is pressure driven in difference from the systems described above.

4.2 Electroporation

Numerous high-resolution techniques exist to detect, image and analyze the biochemical contents of single cells and organelles, few methods exist to control and selectively manipulate the biochemical nature of these compartments. The plasma lipid membrane surrounding cells is impermeable to most compounds of biological and medical interest (e.g. dyes, drugs,

DNA, RNA, proteins, peptides, and amino acids). Thus, to introduce or withdraw such compounds from the cell the bilayer membrane has to be broken. Electroporation is a non-contact method for transient permeabilization of cells using high electric field pulses. Compared to commercial equipment, a flow-type electroporation microchip overcomes the limit in the amount of target cells and the potential risk of using high voltage, which are the two drawbacks in current electroporation technology. One electroporation chip has been reported on by Lin *et al.* [58] where the chip consists of a microchannel in plastic with gold thin film electrodes on both sides. The experimental results showed that electrical pulses with a significantly lower applied voltage could help to deliver reporter genes into Huh-7 cells in continuous manner.

A silicon microteeth device that open and close like jaws to harmlessly deform cells has been developed by Sandia National Laboratories which is shown in figure 4 [59]. The microjaws fit in a 20 µm wide microchannel and puncture cells at the rate of 10 cells a second. The ultimate goal is to replace the microteeth with hollow silicon needles to puncture cells and inject them with DNA, proteins, or pharmaceuticals at precise points of cells and in large numbers, possibly changing the course of a disease or restoring lost functions. However, one can also imagine that the device could be used for cell lysis. Another injection system which consists of two components; hollow microneedles for injection and microchambers for cell trapping has been reported by Chun *et al.* [60]. Another example is presented by Zappe *et al.* who used a surface micromachined needle for injecting dsRNA into embryonic cells [61].

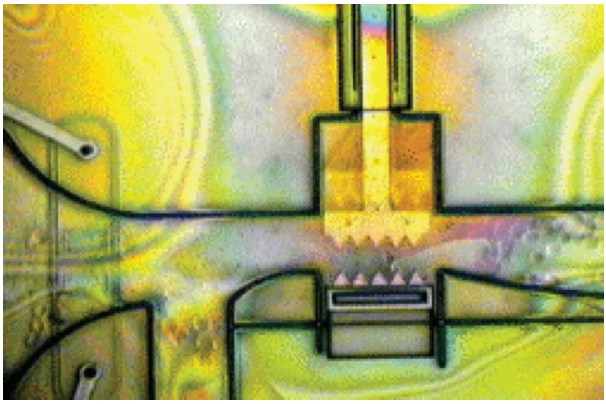


Figure 1-4. A silicon microteeth device with microjaws that harmlessly deform cells (www.sandia.gov).

The first system for single cell electroporation was presented by Rubinsky [62-64]. The chip is a three-layer device that consists of two translucent polysilicon electrodes and a silicon nitride membrane, which together form two fluid chambers. The two chambers are interconnected through a single micron size hole in the insulating silicon nitride membrane. In a typical process, the two chambers are filled with conductive ionic solutions. One chamber contains cells; individual cells can be captured in the hole and thus incorporated in the electrical circuit between two electrodes of the chip. Experiments show that the chip has the capability to manipulate and induce electroporation on specific individual cells. As indicated in a recent review on single-cell electroporation, [65], single-cell electroporation makes it possible to investigate cell-to-cell variations in a population and to manipulate as well as investigate the intracellular chemistry of a cell. Further miniaturization of the electrodes to the nanoscale will allow selective manipulation of single organelles within a cell. Another possibility is to combine electroporation with analytical techniques such as capillary electrophoresis separation and mass spectroscopy to perform single cell proteomic studies.

4.3 Cell Fusion

There are a number of methods for carrying out cell-cell fusion *in vitro*, including the use of chemicals, the use of focused laser beams and the application of pulsed electric fields (electrofusion). Of these methods electrofusion has gained popularity because of its ease of implementation, high efficiency, and reproducibility. To carry out electrofusion, a suspension of cells in a fusion chamber is first brought into physical contact by dielectrophoresis using a low amplitude, high frequency AC field. Subsequent application of a short duration high intensity electric pulse then causes a fraction of cells that are in close contact to fuse. The ease with which arrays of microelectrodes can be patterned and integrated with networks of microchannels makes microfluidic systems a particularly attractive platform for applications in electrofusion in which fusion among a multitude of different cell types is desired [66-67]. Strömberg *et al.* has for example demonstrated electrofusion of single pairs of cells in a microfluidic device [67]. Hence, enabling the controlled combination of any two cells with similar appearance but, for example, different genetic composition. This technique may be useful in the production of hybridomas, cloning, and studies of genetic expression in the future. Integrated microelectrode microfluidic systems, in addition to providing versatility for manipulating and transporting cells, avoid the necessity for expensive high voltage pulse generators, which offers the possibility of a cheap and disposable platform.

5. CELL ANALYSIS

Performing cellular assays in microfluidic devices provides the advantages of reduced cell consumption, automated reagent addition, and reproducible mixing of reagents with cells.

5.1 Cell Transport and Cultivation

A microfluidic device for on-chip monitoring of cellular reactions has been developed by Yang *et al.* [68]. The device consists of two primary analytical functions: control of cell transport and immobilization, and dilution of an analyte solution to generate a concentration gradient. A dam structure in parallel to the fluid flow was constructed for docking and alignment of the cells. The structure allows the cells to move in the microfluidic channels and dock in desired locations with controllable number. An analyte solution could be diluted to different gradients as a function of distance along the dam. The ATP-dependent calcium uptake reaction of HL-60 cells was used as a model for on-chip measurement of the threshold ATP concentration that induces significant intracellular calcium signal. The results demonstrated the feasibility of using the microchip for real-time monitoring of cellular processes upon treatment of a concentration gradient of test solution. Ahn *et al.*. has presented a polymeric device for metabolic monitoring of human islet cells. The microfluidic system, integrated with a glucose sensor for simultaneous glucose measurements, entraps and sustains pancreatic islet cells in a micro reaction chamber [69].

Livestock embryo manipulation is becoming more a routine due to the development of gene manipulation, cloning, and in vitro fertilization techniques. This requires the handling of individual embryos. Glasgow have designed and tested a microfluidic system capable of transporting individual, pre-implantation mouse embryos through a network of channels to selected locations [70]. The flow of the buffer causes the embryos to roll down the channels. Embryos can be spatially retained and then released or moved to a new location via the manipulation of flows.

Sperm motility studies and sperm selection was performed in a microfluidic channel device by Kricka *et al.* [71]. Semen was also tested for the presence of sperm-specific antibodies by using microchannels filled with human anti-IgG antibody coated beads. Tracey *et al.* have developed a device for cytomechanical studies of red blood cell membrane viscoelastic behavior during flow in microfluidic channels [72].

Structures with micrometer-sized features and patterns are useful in manipulating cells and studying the effect of microenvironments on cell behavior. Cell shape affects cell growth, gene expression, extracellular

matrix metabolism, and differentiation. Thus, topographical features that can regulate cell shape have potential applications in the study of fundamental cell biology. Takayama *et al.* [73] tested some topographically patterned capillaries as chambers for mammalian cell culture. Cells cultured inside capillaries with topographical features elongate along the grooves and the ridges of the topography. Cells cultured inside capillaries with flat surfaces spread equally in all directions. Turner *et al.* [74] have studied the attachment of astroglial cells on smooth silicon and arrays of silicon pillars and wells with various widths and separations. Fluorescence, reflectance and confocal light microscopies as well as scanning electron microscopy were used to quantified cell attachment, describe cell morphologies and study the distribution of cytoskeletal proteins actin and vinculin on the different surfaces. The results support the use of surface topography to direct the attachment, growth and morphology of the cells. Microfluidic shear devices for studying cell adhesion on biological substrates have been presented by Lu *et al.* [75]. The device design takes advantage of laminar flow in microfluidic systems to induce steady shear stresses on adherent cells.

A microfluidic device that permits growth of bacteria on chip has been presented by Monaghan. *et al.* [76]. The device consists of a microfabricated capillary in PDMS. The growth of *E.coli* was monitored over a 5 h period visually and by fluorescence. This device has several advantages over their traditional counterparts, including real-time monitoring, increased sampling frequency, automation and reduction of biological specimen volume and waste. Szita *et al.* [77] have presented a batch microfermentor with a working volume of 5 µl with integrated sensors for the measurement of dissolved oxygen and pH. Growth behavior as well as the curves of DO and pH are comparable to results obtained from conventional 500 ml batch fermentors.

Another microfluidic device in PDMS consisting of an eight lines micro-injector array integrated in a base flow channel has been realized by Thiebaud *et al.* [78]. This system allows controlled application of drugs to patterned (by microcontact printing) cell cultures. Walker *et al.* presented a device consisting of microfluidic channels out of PDMS that were used as culture vessel for ovary cells. PDMS allows cells to be visually inspected and provides excellent permeability to oxygen and carbon dioxide [79].

Tamaki *et al.* has developed a microsystem for cell experiments consisting of a scanning thermal lens microscope detection system and a cell culture microchip [80]. The microchip system was good for liquid control in microspace and this results in secure cell stimulation and coincident in vivo observation of the cell responses. The system could detect nonfluorescent biological substances with high sensitivity. The system was applied to

monitoring of cytochrome c distribution in a neuroblastoma-glioma hybrid cell cultured in a microflask fabricated in a glass microchip.

Yasuda *et al.* has presented a system for continuous observation of isolated single cells which enables genetically identical cells to be compared using an on-chip microculture chip and optical tweezers [81]. The microchambers are connected by a channel through which cells are transported by the optical tweezers from a cultivation microchamber to an analysis chamber, or from the analysis chamber to a waste chamber. Differential analysis of isolated direct descendants of single cells showed that this system could be used to compare genetically identical cells helping to explain heterogeneous phenomena.

Microarrays of selectively localized living cells containing engineered fluorescent protein biosensors for high throughput screening have been presented by Kapur *et al.* [82]. HTS 'hits' are identified using one biosensor while reading the whole chip array of cells. Biological information is obtained from probing target activity at intercellular, sub-cellular and molecular levels in the 'hit' wells. The miniaturized platform consists of single or multiple engineered cell types microarrayed in predetermined spatial addresses on an optically clear substrate. Self-assembled hetero-monolayers coupled with arrayed cell-specific ligands are used to array singled or multiple cell types.

Caliper Technologies/Agilent has designed capillary electrophoresis chips for cell assays such as fluorescently labeled annexin-V detection for apoptosis, as well as monitoring of transfection efficiency by GFP detection or antibody staining. The cell fluorescence LabChip Kit provides special chips and reagents that allow analysis up to six pre-stained cell samples per chip. In Caliper's format the cells are flowed continuously in microchannels, reagents are added sequentially, and cell response is measured non-intrusively by fluorescence. Cell consumption as low as 50-100 cells per assay yields excellent screening results.

5.2 Electrical Characterization

The use of cell-based biosensors outside of the laboratory has been limited due to many issues including preparation of the sample, maintenance of the biological environment, and integration of the electronics for data collection and analysis. DeBusschere *et al.* has described a system that addresses several of these issues with the development of an integrated silicon-PDMS cell cartridge [83]. The cell cartridge contains a CMOS silicon chip that incorporates a digital interface, temperature control system, microelectrode electrophysiology sensors, and analog signal buffering.

A microsystem for the culture and electrical characterization of epithelial cell layers has been developed by Hediger *et al.* [84-85]. The main goal was to achieve both cell culture and impedimetric and potentiometric characterization on a single device. A microchip with buried microchannels for culture, stimulation and recording of neural cells was presented by Heuschkel *et al.* [86]. The microchannels allowed for local and fast delivery of drugs to the cells. A microsystem that consists of a two-microelectrode sensors integrated with high aspect ratio chambers (360 pl) made of SU8 was presented by Cai *et al.* [87]. Dynamic electrochemical measurements of lactate during cell permeabilization of single heart cells were performed.

5.2.1 Patch Clamp on Chip

Chip based patch clamping has the objective to replace traditional patch electrodes with a planar array of recording interfaces miniaturized on the surface of either a silicon, polymer or glass substrate. One chip-based device for patch clamping was presented by Schmidt *et al.* [88], which consists of planar insulating diaphragms on silicon. In this work it is shown that stable gigaohm seals over micrometer-sized holes can be obtained in the time frame of seconds by electrophoretic self-positioning of charged lipid membranes. Recording chips can be produced in large numbers with defined geometry and material properties by standard silicon technology. Multiple recording sites can be integrated on one single chip because of the small lateral size of the diaphragms.

Three dimensional silicon oxide micro-nozzles integrated into a fluidic device for patch clamping has been developed by Lehnert *et al.* [89]. A cell can be positioned on the nozzle by suction through the hollow nozzle that extends to the backside opening of the chip. A microanalysis system for multi purpose electrophysiological analyses has been presented by Han *et al.* [90]. This system has the capability to perform whole cell patch clamping, impedance spectroscopy, and general extracellular stimulation/recording using integrated, multi-electrode configurations.

The loss of physical integrity in the plasma membrane is one of the major indications of cell death. Cell viability is thus usually determined through examination of membrane integrity with colorometic of fluorescent dues. Huang *et al*. have developed a new technology that employs a microfabricated device for high-resolution, real-time evaluation of membrane electrical properties of single cells [91]. The chip allows probing a single cell with low electrical potentials without introducing membrane damage, and measuring of the corresponding electrical currents flow through that cell. Electrical resistances of dead (membrane impaired) cells and live cells were found to be significantly different. This suggests that evaluating

membrane resistances of individual cells can provide an instant and quantitative measure to determine cell membrane integrity and cell viability of single cells.

5.3 Mechanical Characterization

Cells in viable tissues respond to mechanical stimuli under physiological and pathophysiological conditions through alterations in the activity of ion channels and the concentrations of signaling molecules, which ultimately lead to modifications of the cytoskeleton and extracellular structures. To begin to understand the complex biological mechanisms that organize cellular response to mechanical forces, many different in vitro devices have been developed to apply static, and dynamic mechanical stimulus to cell culture [92]. Micromechanical systems (MEMS) technology offers the ability to shrink the entire force transducer down to a size comparable to that of a single cell. A fully submersible force transducer system has been implemented using MEMS designed for use with single, living heart cells [93]. The scale of the device works well for the study of many cell types whose dimensions lie in the 25-250 μm range. The cell force transducer was successfully operated with cardiac myocytes in a saline bath surviving multiple solution exchanges under steady state and oscillatory conditions [93].

5.4 (Bio)chemical Sensing

(Sub)micron size biochemical sensors and electrodes can be used for analysis of intracellular parameters (pH, conductivity) as well as the presence of cell metabolites (e.g. calcium). The electrochemical signature of peroxynitrite oxidation, an important biologically active species, has been studied using microelectrodes on single cell level [94]. A method for preparing Pt electrodes with nanometer dimension has been reported by [95], demonstrating the ability to voltammetrically detect zeptomole quantities of an electroactive species. Recently an attempt has been reported to make a micro-ion sensor array to determine intracellular ion concentrations [96].

6. CONCLUSIONS

After having proven the value of microfluidics for genetic and proteomic analysis, this chapter illustrates it is also a very useful concept for cell analysis. Most of the cited work derives from the past 5 years, with a clear trend towards single cell analysis. Hence, the most important development in

cellomics is the possibility to treat and analyze single living cells. It is clear that with the recent technological developments many life-sciences researchers obtain a very powerful tool for detailed cellular studies. We anticipate an even further growth of interest when also nanotechnological tools are included in this area. Undoubtedly, microfluidic devices for cellomics will generate a large variety of new and exciting research and development topics.

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