Lab-in-a-cell: Using Individual Cells as Experimentation Platforms

Helene Andersson[1,2,3] and Albert van den Berg[1]

[1]MESA⁺ Institute, University of Twente
BIOS, the Lab-on-a-Chip Group
P.O. Box 217
7500 AE Enschede, The Netherlands

[2]Royal Institute of Technology, Department of Signals, Sensors and Systems,
100 44 Stockholm, Sweden

[3]Silex Microsystems AB, 175 26 Jarfalla, Sweden

Abstract There are many efforts today trying to mimic the properties of single cells in order to design chips that are as efficient as cells. However, cells are nature's nanotechnology engineering at the scale of atoms and molecules. Therefore, it might be better to envision a microchip that utilizes a single cell as an experimentation platform. A novel so-called Lab-in-a-Cell (LIC) concept is described, where advantage is taken of micro/nanotechnological tools to enable precise control of the biochemical cellular environment and the possibility to analyze the composition of single cells.

Introduction

The understanding of many biological processes would benefit greatly from the ability to analyze the content of single cells. Today, there are only a few conventional systems available that enable direct intrinsic studies of single cells, including capillary electrophoresis, patch clamping and flow cytometry [1]. These systems, however, are based upon conventional technologies and instrumentation, give only limited information about the cell content and do not present a general method for single cell analysis. The recent rapid developments in micro-and nanofabrication technologies, which have already led to the successful so-called 'Lab-on-a-chip' (LOC) concept, also open up great opportunities for analysis of single cells.

The 'Lab-on-a-chip' (LOC) or 'Micro Total Analysis Systems (μTAS) concept has received rapidly growing interest in the past ten years, as illustrated by several

review articles which go into more or less detail [2-6]. Initially, there were two approaches followed in this field, the first aiming at combining microsensors with fluidic components (pumps, flow sensors) into systems (*e.g.* ammonia/phosphate sensing) [7-8]. The second approach, which had a much greater impact, focused on miniaturization of analytical chemical methods, in particular separations, with (after the first demonstration with amino acids [9]) a lot of emphasis on genetic (DNA) analysis [10-13]. In Figure 1 an example of a lab-on-chip for genetic analysis is shown [13]. 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 [3].
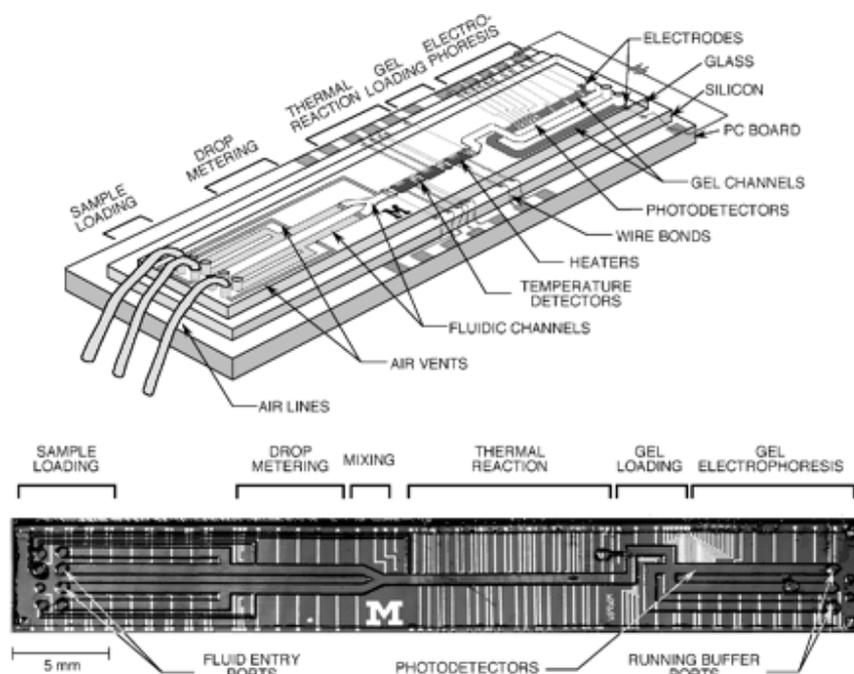

Figure 1. (Top) Schematic of integrated device with two liquid samples and electrophoresis gel present. (Bottom) Optical micrograph of the device from above. Reproduced from [13] with permission.

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. A recent review illustrates the fact that microtechnologies are very useful for cell manipulation and analysis [14], and that they have advanced to a level that allows control of mechanical, electrical and biochemical parameters down to the nanometer scale. Most of the cited work derives from the past 5 years, with a clear trend towards single cell analysis, as

illustrated by the development of chip-based devices for single cell ion channel studies [15-17]. Although no reports of direct chemical analysis of single cells in microfluidic devices are known, Waters *et al.* have used microstructures for cell lysis, followed by PCR and capillary electrophoresis (CE) for analysis of the DNA of cells [18]. Nevertheless, cell analysis even without need for amplification seems only to be a question of time, as recently new approaches to single cell analysis by CE have been proposed by Zabzdyr *et al.*, albeit in a conventional system [19].

It is clear that with the recent technological developments many life-science researchers obtain very powerful tools for detailed cellular studies [20]. The novel concept, Lab-In-a-Cell (LIC), described in this article intends to combine the best of both worlds by using the biological "unit" - a cell - as a laboratory in which to perform complex biochemical operations, and by using advanced micro- and nanotechnological tools to access and analyze this laboratory and interface it with the outside world. This idea of combining efficient and specific functioning, generated by "natural" structures, with man-made micro/nanofabricated devices has similarities with other examples such as the use of the natural ionophores (*e.g.* the antibiotic valinomycin) to create highly selective ion sensors [21], entrapment of enzymes such as glucose oxidase for obtaining a micro-glucose sensor [22], or utilization ion-channel proteins (*e.g.* α-hemolysin) incorporated in lipid bilayers for single molecule DNA sequencing [23], as well as in the concept of cell-based sensors by [24]. The LIC concept, however, aims at exploiting the incredible complexity and effectiveness of individual cellular processes in a much broader scope, as we will illustrate below.

Concept

In biological systems, such as single cells, the parallel handling of small numbers of molecules is inherent. A differentiated eucaryotic cell can perform some 10^3-10^4 different chemical operations simultaneously, depending on the protein content, at overall burning rate of 10^6 molecules/s of ATP [25]. All this is performed in a volume element in the order of 1 picoliter. How is this chemical multiprocessing capability in extremely small volumes possible? Part of the solution to this question is found in five main features: 1) compartmentalization (*i.e.* the usage of specialized reaction containers such as organelles with volumes of 10^{-15}-10^{-21} liter, with controlled input and output properties), 2) molecular recognition (highly specific interactions between reacting molecules or binding interactions in a sorting/counting step), 3) a combination of small scale and complex function, 4) targeted transport and controlled mixing of components (for example by the use of vesicles as cargo carriers between organelles) [25], 5) preservation of internal laboratory conditions. There are many efforts today trying to mimic these properties of single cells in order to design chips that are as efficient as cells. We believe it might be better to take advantage of the optimized

natural "laboratory" represented by a cell and to envision a chip where a single cell constitutes the core, a Lab-In-a-Cell.

Imagine that today's life science laboratories are represented by single cells on chip. Then you would need several different components on the chip in order to put your 'lab' on a specific position on the chip, to study what is going on inside the 'lab', to deliver and/or withdraw data from it (see Figure 2). Hence, for the new LIC concept the following functions have to be provided:

- Cell manipulation/immobilization
- Electrical/mechanical/biochemical/optical characterization
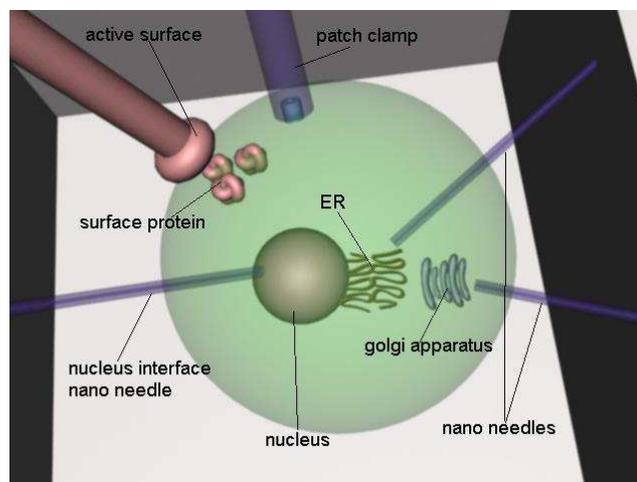- Connection/communication external/internal

Figure 2. Conceptual drawing of the lab-in-a-cell concept.

In the literature up to the present a few examples have been presented for single cell analysis on chip [26]. In figure 3, one example of LIC is shown; here single cells are trapped on chip and enable real time studies of apoptosis which is not possible today with the conventional technologies [27].
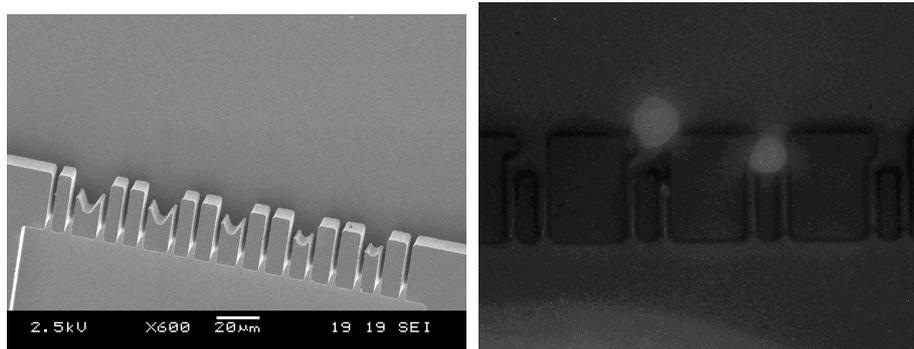
Figure 3. A single cell trap for real time studies of cellular processes [27].

Discussion

Populations of cells have been used as 'workhorses' for producing biological compounds or as sensing elements since the beginning of 20th century. However, it has not been possible to access and make use of the processes of individual cells due to the lack of tools able to operate on and interact with individual cells. New micro/nanotechnological tools have now opened new opportunities for realizing novel biochemical/mechanical/optical-characterization methods of single cells.

Possible applications of LIC systems are for example 1) detailed studies of intracellular processes and mechanisms, *e.g*. detecting ion-channel responses as function of external stimuli, 2) use of single cells as nanoreactors for combinatorial chemistry, 3) use of single cells as platform for drug testing (thereby reducing the need of animal testing) and 4) the development of single cell based sensors etc.

Hopefully, this article promotes awareness among biologists that single cells can be considered as experimental platforms, as well as acting as a stimulus for micro/nano engineers to further develop and refine the needed instrumentation.

Conclusion

Currently much effort is being put into trying to mimic the properties of single cells in order to design chips that are as efficient as cells. However, cells are nature's nanotechnology engineering at the scale of atoms and molecules and it will be very difficult (impossible) to create lab-on-chips that are as efficient as cells. Therefore, it might be better to envision chip solutions where a single cell constitutes the core, the workhorse, and the chip is the interface that enables manipulation, characterization and communication.

Acknowledgements

References

1. Krylov, S. and Dovichi N: Single-cell analysis using capillary electrophoresis: Influence of surface support properties on cell injection into the capillary, *Electrophoresis* 2000, 21:767-773.
2. Reyes, D., D. Iossifidis, P. Auroux and A. Manz: Micro total analysis systems. 1. Introduction, Theory, and Technology, *Analytical Chemistry* 2002, 74:2623-2636.
3. Auroux, P., D. Iossifidis, D. Reyes and A. Manz: Micro total analysis systems. 2. *Analytical standard operations and applications, Analytical Chemistry* 2002, 74: 2637-2652.
4. Tudos, A., G. Besselink and R. Schasfoort: Trends in miniaturized total analysis systems for point-of-care testing in clinical chemistry, *Lab on a chip 2001*, 1: 83-95.
5. Verpoorte, E: Microfluidic chips for clinical and forensic analysis, *Electrophoresis 2001,* 23:677-712.
6. van den Berg, A. and T. Lammerink: Micro total analysis systems: Microfluidic aspects, Integration concept and applications, *Topics in Current Chemistry* 1998, 194:21-49.
7. Darion, A., M. Sequeira, G. Pennarun-Thomas, H. Dirac, J. Krog, P. Gravensen, J. Lichtenberg, D. Diamond, E. Verpoorte and N. de Rooij: Chemical sensing using an integrated microfluidic system based on the Berthelot reaction, *Sensors and Actuators B* 2001, 76:235-243.
8. Verpoorte, E., B. van der Schoot, S. Jeanneret and A. Manz: 3-dimensional micro-flow manifolds for miniaturized chemical analysis systems, *J. of Micromechanics and microengineering* 1994, 4:246-256.
9. Harrison, J., K. Fluri, K. Seiler, Z. Fan, C. Effenhauser and A. Manz: Micromachning a miniaturized capillary electrophoresis-based chemical analysis system on chip, *Science* 1993, 261:895-897.
10. Liu, S., Y. Shi, W.W. Ja and R.A. Mathies: Optimization of high-speed DNA sequencing on microfabricated capillary electrophoresis channels, *Analytical Chemistry* 1999, 71:566-73.
11. Mathies, R., P. Simpson and A. Woolley: DNA analysis with capillary array electrophoresis microplates, *MicroTAS* 1998, Banff, Canada, 1-6.
12. Simpson, P.C., D. Roach, A.T. Woolley, T. Thorsen, R. Johnston, G.F. Sensabaugh and R.A. Mathies: High-throughput genetic analysis using microfabricated 96-sample capillary array electrophoresis microplates, *Proc. Natl. Acad. Sci. U S A* 1998, 95:2256-61.
13. Burns, M., Johnson, B., Brahmasandra, S., Handique, K., Webster, J., Krishnan, M., Sammarco, T., Man, P., Jones, D., Heldsinger, D., Mastrangelo, C., Burke, D.: An integrated nanoliter DNA analysis device, *Science* 1998, 282:484-487.
14. Andersson, H., van den Berg, A: Microfluidic devices for cellomics: a review, *Sensors and actuators B* 2003, 92:315 – 325.
15. Schmidt, C., M. Mayer and H. Vogel: A chip-based biosensor for the functional analysis of single ion channels, *Angew. Chem. Int*. 2000, 39:3137-3140.

16. Lehnert, T., M. A. M. Gijs, R. Netzer and U. Bischoff: Realization of hollow SiO2 micronozzles for electrical measurements on living cells, *Appl. Phys. Lett.* 2002, 81:24.
17. Han, A., E. Moss, R. Rabbitt and B. Frazier: A multi-purpose micro system for electrophysiological analyses of single cells, *microTAS* 2002, 805-807.
18. Waters, L.C., S.C. Jacobson, N. Kroutchinina, J. Khandurina, R.S. Foote and J.M. Ramsey: Microchip device for cell lysis, multiplex PCR amplification, and electrophoretic sizing, *Anal. Chem.* 1998, 70:158-62.
19. Zabzdyr, J. and S. Lillard: Measurement of single-cell gene expression using capillary electrophoresis, *Trends in Analytical Chemistry* 2001, 20:467-476.
20. Roco, M: Nanotechnology: convergence with modern biology and medicine, *Current opinion Biotech* 2003, 14:337-346.
21. D. Ammann: Ion *Selective Microelectrodes: Principles, design and Applications*, Springer Verlag, Berlin, 1986.
22. M. Koudelka-Hep, D.J. Strike and N.F. de Rooij: Miniature electrochemical glucose biosensors, *Anal. Chim. Acta* 1993, 281:461-466.
23. Kasianowicz, J., Brandin, E., Branton, D., and Deamer, D.: Characterization of individual polynucleotide molecules using a membrane channel, *Proc. Natl. Ac. Sci. USA* 1996, 93:13770-13773.
24. L. Bousse: Whole cell biosensors, *Sensors and Actuators B* 1996, 34:270-275.
25. Davidson, M., Karlsson, M., Sinclair, J., Sott, K., Orwar, O.: Nanotube-vesicle networks with functionalized membranes and interiors, *J. Am. Chem. Soc.* 2002, 125:374-378.
26. Andersson, H., van den Berg, A., Micro and nanotechnologies for single cell analysis, *Current Opinion on Biotechnology,* 2004*, 15, 1-6.*
27. A. Valero, F. Merino, F. Wolbers, R. Luttge, I. Vermes, H. Andersson and A. van den Berg, Apoptotic cell death kinetics of HL60 cells studied using a microfluidic cell trap device, *Lab on chip*, in press.