with a detection limit of 0.4 mmol/l. The measurement error in the size of the peak area does not sufficiently explain the error of approximately 10% on the recovery for the serum sample. Depending on the choice of buffer system the concentration profile of ions in the sample plug may be influenced by the matrix composition of sample and does not necessarily represent the original concentration. To investigate the sources of error in detail more experiments are necessary.

Conclusion

The experiments demonstrated that lithium was separated from a drop of whole blood with capillary electrophoresis on a microchip within two minutes. Currently further investigations are conducted studying the process of concentration adjustment during the sample loading in order to improve on the accuracy for quantitation. In addition, potassium was detected by this method and more study is currently dedicated to separate calcium and magnesium in order to utilize the full potential of these microchips for 'point of care'-testing.

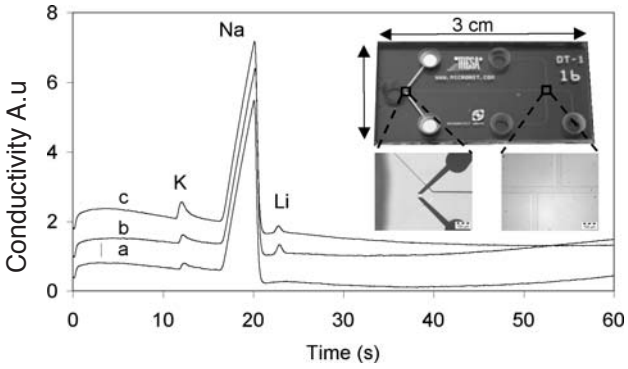

Figure 1. Results of a separation of a) whole blood without lithium, b) whole blood spiked with 2 mmol/l lithium and c) blood serum spiked with 2 mmol/l lithium. The inset shows a photograph of the CE chip with a capillary length of 2 cm (Micronit Microfluidics, The Netherlands) with a blow-up of the end-column conductivity detection electrodes and the double-T injection region defining the size of the sample plug dispensed into the separation channel.

References

1. Verpoorte E. Electrophoresis, 2002; 23: 677-712.
2. Berg A van den, Lammerink TSJ. Topics Curr Chem 1998; 194: 21-37.
3. Guij RM, Baltussen E, Steen G van der, Schasfoort RBM, Schlautmann S, Billiet HAH, Frank J, Dedem GWK van, Berg A van den. Electrophoresis 2001; 22: 235-241.
4. Pumera M, Wang J, Opekar F, Jelinek I, Feldman J, Lowe H, Hardt S. Anal Chem 2002; 74: 1968-1971.
5. Lichtenberg J, Rooij NF de, Verpoorte E. Electrophoresis 2002; 23: 3769-3780.
6. Tanyanyiwa J, Abad-Villar EM, Fernandez-Abedul MT, Costa-Garcia A, Hoffmann W, Guber AE, Herrmann D, Gerlach A, Gottschlich N, Hauser PC. Analyst 2003; 128: 1019-1022.
7. Berthold A, Laugere F, Schellevis H, Boer CR de, Laros M, Guijt RM, Sarro PM, Vellekoop MJ. Eelectrophoresis 2002; 23: 3511-3519.
8. Hjertén S. J Chromatogr 1985; 347: 191-198.
9. Vrouwe EX, Luttge R, Berg A van den. Electrophoresis, accepted for publication.

Ned Tijdschr Klin Chem Labgeneesk 2004; 29: 296-297

Apoptosis induced kinetic changes in autofluorescence of HL60 cells – application for single cell analysis on chip

F. WOLBERS[1,2], A. VALERO[2], H. ANDERSSON[2], R. LUTTGE[2], A. van den BERG[2] and I. VERMES[1]

Introduction

Natural cellular autofluorescence (AF) can be a useful tool to unravel intracellular pathophysiological processes and distinguish normal from diseased tissue. Many cellular metabolites exhibit autofluorescence, e.g. NAD(P)H and flavins, which colocalizes strongly within the mitochondria and in some extent to the lysosomes (1-6). Both components are actively involved in a number of metabolic processes within the cell and play an important role in the energy household of the cell. This paper presents a new method using AF to study apoptosis. Apoptosis or programmed cell death plays an important role in maintaining a homeostatic equilibrium between cell proliferation and cell death. Induction of apoptosis results in shrinkage of the cell and fragmentation into apoptotic bodies (7). AF intensity is first measured conventionally at the flow cytometer (FCM) and finally the results will be translated on to a microfluidic chip to perform single-cell analysis.

Department of Clinical Chemistry[1], Medisch Spectrum Twente, Hospital Group and Department of Sensorsystems for Biomedical and Environmental Applications[2], MESA+ Institute, University of Twente, Enschede, The Netherlands

Autofluorescence measurements

Human promyelocytic leukemic HL60 cells were incubated with camptothecin (CPT), tumour necrosis factor (TNF)-a in combination with cycloheximide (CHX), or irradiated with 6 or 10 Gy, during varying

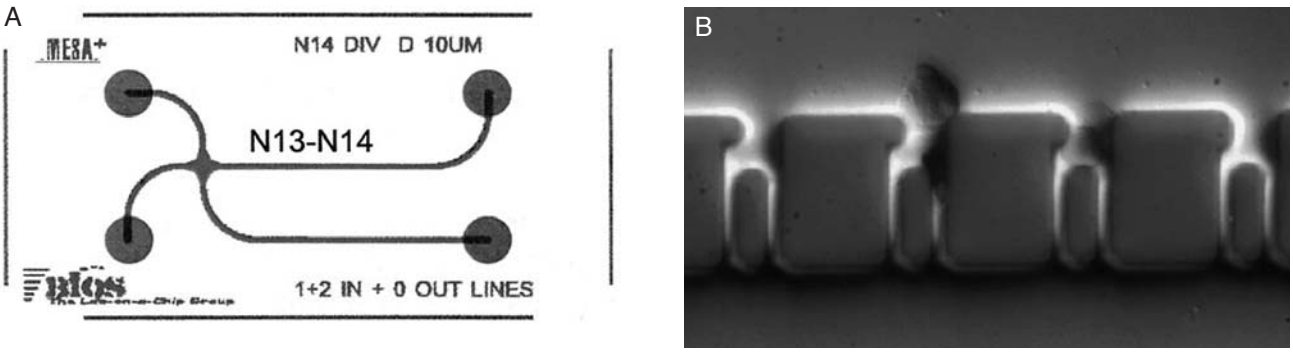

Figure 1. A. Microfluidic chip for trapping cells. B. Trapped HL60 cells in the microfluidic chip.

time periods, to initiate apoptosis. AF was measured using the FL-1 (CD103F, 525 nm) channel at the FCM. Induction of apoptosis results in the shrinkage of the cell and the fragmentation into apoptotic bodies. With flow cytometry, 4 subpopulations can be distinguished, viable, early apoptotic, late apoptotic and the necrotic population, based on differences in size and contents of the cell (forward- and sideward scatter), corresponding to the different stages of the apoptotic cascade in vitro. Induction of apoptosis results in a decrease in AF intensity compared to untreated HL60 cells. The decrease in AF intensity is especially seen in the late apoptotic cells. To translate these effects to a microchip, the change in AF intensity from 2h to 24h ($AF^{24/2}$ factor), corresponding to the maximal increase and minimal decrease, respectively, in AF intensity, is measured (table 1). For all the four inducers the $AF^{24/2}$ factor is decreased, compared to untreated HL60 cells (control). Untreated HL60 cells show only minor fluctuations in time and can be seen as a stable population. Therefore the $AF^{24/2}$ factor for untreated HL60 cells is set at 1.

Cell handling on a microfluidic chip

Recent results have shown that it is possible to perform AF measurements on single cells in a microfluidic device (8). Here a new microfluidic cell assay has been developed enabling the capture of viable cells (figure 1). Once cells go into apoptosis their mechanical properties, e.g. size, change and these apoptotic cells are able to pass the capture position. A decrease in AF intensity of the cells that passed the trap will confirm this hypothesis. In future developments the optical detection will be transferred to an electrical on-chip cell counter specific for apoptosis.
We can speculate that this microfluidic device specific for measuring apoptosis could be a suitable tool for pharmacological studies investigating the effect of various drug treatments on apoptotic cell death.

Table 1. AF 24/2 factor of untreated HL60 cells (control) vs HL60 cells incubated with the different apoptotic inducers used. AF24/2 factor defines the ratio of the maximal AF intensity (t=2h) compared to the minimal AF intensity (t=24h).

	Control	6Gy	10Gy	TNF/CHX	CPT
AF 24/2 factor	1.0	0.68	0.72	0.65	0.68

Acknowledgements

Financial support from STW and valuable work of Jurjen Emmelkamp, Nicolas Demierre and Roy de Kinkelder is gratefully acknowledged.

References

1. Dellinger M, Geze M, Santus R, Kohen E, Kohen C, Hirschberg J-G, Monti M. Imaging of cells by autofluorescence: a new tool in the probing of biopharmaceutical effects at the intracellular level. Biotechnol Appl Biochem 1998; 28: 25-32.
2. Knight A-W, Billinton N. Distinguishing GFP from cellular autofluorescence. Biophotonics Int 2001.
3. Aubin J-E. Autofluorescence of viable cultured mammalian cells. J Histochem Cytochem 1979; 27: 36-43.
4. Petty H-R, Worth R-G, Kindzelskii A-L. Imaging sustained dissipative patterns in the metabolism of individual living cells. Physical Review Lett 2000; 84: 2754-2757.
5. Brock R, Hink M-A, Jovin T-M. Fluorescence correlation microscopy of cells in the presence of autofluorescence. Biophys J 1998; 75: 2547-2557.
6. Benson R-C, Meyer A, Zaruba M-E, McKhann G-M. Cellular autofluorescence-Is it due to flavins? J Histochem Cytochem 1979; 27: 44-48.
7. Vermes I, Haanen C, Reutelingsperger C. Flow cytometry of apoptotic cell death. J Immunoll Meth 2000; 243: 167-190.
8. Emmelkamp J, Wolbers F, Andersson H, Da Costa R-S, Wilson B-C, Vermes I, Berg A van den. Autofluorescence of single living cells for label-free cell sorting in microfluidic system. Submitted to Lab-on-chip.