On-chip non-invasive and label-free cell discrimination by impedance spectroscopy

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Abstract. *Objectives:* Many flow-cytometric cell characterization methods require costly markers and colour reagents. We present here a novel device for cell discrimination based on impedance measurement of electrical cell properties in a microfluidic chip, without the need of extensive sample preparation steps and the requirement of labelling dyes. *Materials and methods, Results:* We demonstrate that in-flow single cell measurements in our microchip allow for discrimination of various cell line types, such as undifferentiated mouse fibroblasts 3T3-L1 and adipocytes on the one hand, or human monocytes and *in vitro* differentiated dendritic cells and macrophages on the other hand. In addition, viability and apoptosis analyses were carried out successfully for Jurkat cell models. Studies on several species, including bacteria or fungi, demonstrate not only the capability to enumerate these cells, but also show that even other microbiological life cycle phases can be visualized. *Conclusions:* These results underline the potential of impedance spectroscopy flow cytometry as a valuable complement to other known cytometers and cell detection systems.

INTRODUCTION

Flow cytometry, like fluorescence-activated cell sorting (FACS), is a powerful and widely accepted approach for single cell analysis. It offers the possibility to analyse several cell parameters simultaneously by antigen and fluorescent labelling and is also capable of sorting the cells. Disadvantages of the method are that the cells get modified permanently and harassed during the labelling procedure, and finally exposed to non-physiological conditions within the cytometer (buffer, hydrodynamic stress). Moreover, most cytometers are expensive, consume costly markers, need well-trained operators, and are not accessible everywhere.

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Figure 1. Electrical model of impedance measurement of a particle, while passing the microfluidic channel with two pairs of superposed electrodes (a and b).

Dielectric measurements like impedance spectroscopy, for characterization of biological tissues and cells, have been well established for about 100 years (Fricke 1924; Schwan 1957; Schanne & P-Ceretti 1978; Pethig & Kell 1987). The most widely used example, the Coulter principle working with direct current (DC) impedance, is applied for counting and sizing single cells and is a component of many blood analysers. Alternating current (AC) impedance measurements provide additional information on cell volume, membrane capacity, and cytoplasmic conductivity or permittivity as a function of frequency (Schwan 1957; Pauly & Schwan 1959; Hoffman & Britt 1979; Hoffman *et al.* 1981). These methods enable discrimination between living and dead cells and monitoring differences in biochemical or morphological structures, without the need for a fluorescent dye or any other additional marker.

The impedance spectroscopy flow cytometer combined with a microchip used in this study allows rapid characterization of cells flowing through a microfluidic channel. The impedance changes over a defined frequency range, when a single cell passes two pairs of superposed electrodes that are aligned in the channel (Fig. 1). By applying frequencies below 1 MHz, the cell membrane forms a barrier to applied electric field, is non-conducting and change of measured impedance is proportional to cell volume. Measurements at intermediate radiofrequencies, which are in the range of the β -dispersion (Schwan 1957; Pethig & Kell 1987), reveal the electric properties of the cell membrane, which becomes permeable for an electric field above 2 MHz. Properties of the cell interior and of cell structure have an influence on the impedance measurements at even higher frequencies (more than 8 MHz), when the membrane is minimally polarized and forms no barrier to the current (Cheung *et al.* 2005). Cell volume influences the impedance measurement to a specific part at all frequencies.

Various cell models were chosen to show functionality of this impedance flow-through cytometer for standard applications, such as haematology, apoptosis, cell differentiation and cell growth. In cell differentiation experiments, in which human blood monocytes were differentiated into dendritic cells, impedance spectroscopy substantiated that this had occurred. The cell types differ from each other in their membrane morphology and to some extent also between cell interiors. To underline also the cytoplasmic properties, mouse fibroblasts were treated with insulin to differentiate them into adipocytes that contain oil droplets in the cell interior. For the distinction of living, dying and dead cells, apoptosis was induced with cycloheximide in Jurkat cells. Apoptosis causes various changes in the cell membrane and cytoplasm. Finally, also to characterize cells from other species, yeast cells were cultured over a longer time period to monitor cell division.

MATERIALS AND METHODS

Cell cultures and treatments

Monocytes and dendritic cells

Monocytes were isolated from human blood buffy coats (SRK Zurich, Switzerland) by Ficoll gradient centrifugation, followed either by surface adhesion to cell culture flasks with complete RPMI medium [10% foetal calf serum (FCS), antibiotics, glutamine, non-essential amino acids, Bioconcept, Allschwil, Switzerland] including 200 U/mL IL-3 for 4 h or by isolating monocytes with CD14-antibodies with magnetic cell separation (MACS, Miltenyi Biotec, Bergisch Gladbach, Germany). Differentiation of monocytes into dendritic cells was induced by adding 200 U/mL IL-4 and 200 U/mL GM-CSF for 6 days and cells were stimulated with 50 ng/mL lipopolysaccharide (LPS) for an additional 2 days to mature. Controls were cultured without any supplements. Cells were collected by centrifugation and were removed from the culture flasks using 5 mM ethylenediaminetetraacetic acid (EDTA)/phosphate-buffered saline (PBS) and diluted in PBS, for measurement.

Differentiation of 3T3 fibroblasts

The mouse embryo fibroblast cell line 3T3-L1 (ECACC, Salisbury, Wilts, UK) was cultured in Dulbecco's modified Eagle's medium with 10% FCS, antibiotics and glutamine (Bioconcept) and was routinely split before reaching 70% confluence. For differentiation of the fibroblasts into adipocytes, the cells were treated 2 days post-confluence with 0.5 mM 3-isobutyl-1-methyl-xanthin (IBMX), 1 μ M dexamethasone and 1 μ g/mL insulin for 2 days and subsequently for a further 10 days with insulin-supplemented medium. Differentiation was controlled visually using oil red O staining. For measurement, cells were trypsinated with 0.5% trypsin/2 mM EDTA (Bioconcept) for 10–20 min and diluted in PBS.

Apoptosis

Jurkat E3.1 cells (ECACC) were continuously cultured in RPMI medium with 10% FCS, glutamine, antibiotics and non-essential amino acids. To induce apoptosis, cells were treated with 0 and 20 μ M cycloheximide (CHX) for 6 h. Cells were afterwards collected for measuring and control staining and were diluted in PBS. Different stainings were performed with DAPI, Annexin-V-FLUOS/PI (Roche Diagnostics GmbH, Mannheim, Germany), and caspase activity was tested with FLICA/SYTOX Green nuclear stain (Image-iT LIVE red poly caspases detection kit, Molecular Probes, Invitrogen, Basel, Switzerland) according to the manufacturers' protocols.

Yeast

Saccharomyces cerevisiae (gift of Prof. Gantenbein, HS Wädenswil, Switzerland) was grown in batch culture in YPD medium. At the starting point, an aliquot of an overnight culture was diluted in fresh YPD. Samples for visual control using microscopy and measurement were taken at 0, 1, 2, 4, 6, 8, 24, 30 and 48 h. Samples for impedance measurement were centrifuged and diluted in PBS.

If not otherwise mentioned, chemicals were purchased at Sigma-Aldrich (Buchs, Switzerland).

Microchip and instrumentation

The microfabricated device was produced as written in Cheung *et al.* (2005) and was placed in a self-fabricated board for electronic and fluid connections. Laminar flow of the cell suspensions was achieved with a miniaturized rotating displacement pump (HNP Microsystems, Parchim, Germany). Cells flowed in the microfluid channel of 20 $\times$ 20 μ m and passed detection electrodes,

which are patterned on the top and bottom surfaces of the channel. The signal was amplified by a connected electronic board which again was coupled with coaxial cables to the following electronic instruments: two function generators 33120A (Agilent Technologies, Irvine, CA, USA) for driving the excitation electrodes of the microchip and two radiofrequency lock-in amplifiers (SR-844 Stanford Research Systems, Sunnyvale, CA, USA; 7280 DSP Signal Recovery, AMETEK, Paoli, PA, USA) for demodulation and amplification of the frequencies. Data acquisition was performed by connecting the lock-in amplifiers with a digital acquisition board DAQ6024E (National Instruments, Austin, TX, USA) to a PC with Matlab software (MathWorks, Natick, MA, USA) for later analysis.

In-flow single cell measurements were always performed at two discrete frequencies that were applied simultaneously. A lower frequency was constantly set at 624 kHz and served as reference, and the second frequency set in the range of 1–15 MHz. For each measurement, normally at least 500 particles were counted. Cells moved at a speed of 2–5 mm per second and were measured at a rate of 200 up to 1000 particles per minute. Thus, the data acquisition programme recorded four channels for every detected particle, each time the real and imaginary part of the impedance signal, presented as amplitude from the lock in amplifiers, for the particular frequency. The impedance signal arises from the difference of the signals that emerge when a cell passes one pair of the electrodes, while at the second electrode pair only medium is present (Fig. 1). From the recorded data, maxima of the signal amplitude were taken, particle speed and combined parameters, such as opacity and ratio, were calculated and plotted. Data are displayed as X- and Y-amplitude of the respective frequency or as X- and Y-ratio, which were calculated as quotient from the particular real and imaginary signal amplitude values of the measurement and reference frequency. These data led to particle size-independent values. Some plots show the 'opacity' as a function of frequency. The electrical opacity of a particle is defined as the ratio of a high-radiofrequency impedance to a low or also DC impedance (Hoffman *et al.* 1981; Coulter Electronics 1988, US patent 4.751.179). Opacity can be used for normalizing cell size of the data and is a measurement of the difference in particle resistivity, which means that parameters other than size become relevant for discrimination. Opacity increases with either decreasing membrane capacitance or decreasing cytoplasm conductivity.

RESULTS

Monocytes and dendritic cells

Ficoll gradient-isolated monocytes were cultured with IL-4 for differentiation into dendritic cells. After maturation with LPS about half of the cells appeared visually as mature dendritic cells (Fig. 2a). The other cells showed a phenotype of intermediary stages or other cell types. In non-supplemented cultures monocytes matured mainly to macrophages. Cell cultures were measured individually and were plotted together in dot plots displaying the X- and Y-ratios (Fig. 2c–e) of the impedance values. Figure 2b shows, for both cell treatments, the mean values of opacity over the frequency. Differentiated dendritic cells can be distinguished from monocytes at middle frequencies (Fig. 2b,d). Both populations show overlapping that is caused by the mixed cell types in the treated cultures.

3T3 fibroblasts and adipocytes

To show functionality of the impedance measurement with another cell differentiation model, mouse fibroblasts (3T3-L1) were treated with insulin after reaching confluence, triggering these

Figure 2. Discrimination of blood monocytes from differentiated dendritic cells. (a) Light microscope photographs of differentiated dendritic cells (left) and macrophages (right), bars 50 μm . (b) Mean values and standard deviation of the opacity over frequency of both cell types. (c–e) Plots displaying the X- and Y-ratios of both cell types at 2, 5 and 14 MHz, where at 5 MHz and partially at 14 MHz separation of both cell populations is visible.

cells to differentiate into adipocytes. The differentiation of the fibroblasts into adipocytes with the formation of the fat droplets was visualized by Oil Red O staining (Fig. 3b). For comparison of differentiated adipocytes to normal fibroblasts, a 3T3-L1 culture, which was just reaching confluence, was selected (Fig. 3a).

Cell cultures were measured individually and are presented together in a plot (Fig. 3c) displaying the mean opacity as a function of the various measurement frequencies. In

Figure 3. Discrimination of 3T3-L1 fibroblasts from differentiated adipocytes. Light microscope photographs of (a) a confluent 3T3-L1 cell culture and (b) 3T3-L1 fibroblasts differentiated into adipocytes, stained with Oil Red O; bars 50 μ m. (c) By displaying the mean and standard deviations of the opacity over frequency a clear discrimination of both cell types becomes evident at middle to high radiofrequencies. (d–f) Plots presenting the X- and Y-ratios of the signal amplitudes show also separation of fibroblasts from the adipocyte cell population at 8 and 14 MHz.

Fig. 3d–f, the X- and Y-ratios of signal amplitudes are plotted from three chosen frequencies to provide a more precise view concerning individual data. Differentiated adipocytes show a higher opacity and can be discriminated from the fibroblastic population at frequencies above 3 MHz.

Figure 4. Control staining of cycloheximide (CHX)-induced apoptosis in Jurkat E3.1 cells. (a, b) DAPI-staining of untreated (a) and with 20 μ M CHX-treated (b) Jurkat cells. Some of the treated cells show characteristic condensed nuclei of apoptotic cells. (c, d) Annexin-V-FLUOS staining of untreated (c) and CHX-treated (d) cells. Apoptotic stages fluoresce green, necrotic cells are counterstained with propidium iodide and appear red. (e–h) Detection of active caspases in untreated (e, g) and CHX-treated (f, h) Jurkat cells with FLICA poly caspases reagent (e, f) and SYTOS Green (g, h) nuclear stain. Cells with active caspases emerge red (e, f). All scale bars are 20 μ m.

Apoptosis

To test operation of the instrument in discrimination of cellular processes, the common model of Jurkat E3.1 cells, a lymphoblastoid line, was chosen and the cells were treated with CHX, to distinguish apoptotic from living cells.

Apoptosis was induced with 20 μ M CHX for 6 h (Gottlieb *et al.* 1996; Tang *et al.* 1999) and then cells were collected for impedance measurement, stained with DAPI, Annexin-V-FLUOS/PI and tested for their caspase activities with FLICA/SYTOS Green nuclear stain (Fig. 4). Stainings indicated that a part of the CHX-treated cells were apoptotic and a few had become necrotic. Impedance measurement plots of signal amplitudes show splitting of the CHX-treated cell cultures at high frequencies (Fig. 5).

Yeast cell growth and division

Saccharomyces was grown in batch culture to examine cell division and growth (Fig. 6). Yeast growth and stadium of cell division (budding) was checked optically using a Neubauer counting chamber. At 1 h and 8 h of culture, around 30% and 50%, respectively, of the cells were budding, compared to 24 h with fewer than 5% budding yeast cells. Measured signal amplitudes show splitting of yeast cells into two populations with comparable percentage separation.

DISCUSSION

In the past years, a transition from high-throughput screening to high-content analysis is being observed and the importance of whole-cell assays is growing steadily. Information gathered from such assays describes complex biological mechanisms that occur in a cell after external stimulation more exactly than single molecular events. Novel technologies, such as bio-MEMS (micro-electro-mechanical system), provide new tools and opportunities to facilitate these

Figure 5. Impedance measurements of Jurkat E3.1 cells induced to apoptosis with cycloheximide (CHX). Plots display signal amplitudes of untreated (red) and CHX-treated (blue) cells at 1 (a) and 12 MHz (b). 12 MHz measurement shows separation of treated Jurkat cells and the lower blue population displays apoptotic cells.

Figure 6. Impedance measurements of yeast cells grown in batch culture over a time course. Plots displaying the signal amplitudes of 1, 8 and 24 h cultures at 8 MHz measurement, plotted individually (a–c) and together (d).

analyses. Impedance spectroscopy flow cytometry has already been shown to represent a valuable alternative to FACS for whole-cell analyses (Cheung *et al.* 2005). Until now, however, only red blood cells have been used, as cell models to reveal the potential of this technology. In this report, we have provided further evidence for the possibility of using flow-through impedance spectroscopy as a universal tool for label-free cell characterization.

Monocytes and dendritic cells

In our study, differentiated dendritic cells could be distinguished from monocytes at middle frequencies (Fig. 2). Decreased opacity of dendritic cells is due mainly to an increase in membrane capacitance as well as to increase in cytoplasmic conductivity which result from their different morphology from leucocytes. Dendritic cells are marked by an irregular cell shape with membrane structures like dendrites or pseudopods. These cell surface structures are more distinct in dendritic cells than they are for monocytes or macrophages. It was shown that membrane capacity was higher with larger membrane area which was caused by increasing concentration of morphological cell surface features (Yang *et al.* 1999; Kiesel *et al.* 2006). The cell interior of dendritic cells is more conductive and is characterized by cytoplasmic possesses, an often contorted nucleus and special organelles such as endosomes, or lysosomes, and large spherical mitochondria (Steinman & Cohn 1973; Hart 1997). Monocytes and macrophages have many lysosomes and vacuoles, and their mitochondria are rode-shaped (Steinman *et al.* 1980). Monitoring dendritic cells grew an increasing interest with the examination of haematopoietic stem cells (Liu & Blom 2000; Arpinati *et al.* 2000), diseases such as arthritis, or control of immunosuppression (Santiago-Schwarz *et al.* 2001; Cravens & Lipsky 2002). We can show with our impedance measurements that dendritic cells can be visualized without extensive antibody marking and detection. Despite overlapping with other cell populations, the measurements provide a first hint for identification and enumeration of dendritic cells in a rapid and simple way.

3T3-L1 fibroblasts and adipocytes

Differentiation of 3T3-L1 fibroblasts into adipocytes was observed at frequencies above 3 MHz (Fig. 3). Differences in the measured impedance and resulting opacity of both populations are caused by differences in membrane capacitance or permeability and are mainly in lower cytoplasmic conductivity. Both membrane and cytoplasm experience changes during the differentiation process. Adipocytes acquire a different morphology from fibroblasts, accumulate triglycerol droplets and have changed enzymatic activity (Green & Meuth 1974; Rubin *et al.* 1977; Smith *et al.* 1986). It is known, for example, that membrane invaginations and intramembrane particles can alter membrane capacitance (Fan *et al.* 1983). Moreover, it was shown that oil droplets in cytoplasm and modified glucose and lipid metabolism (Wiese *et al.* 1995; Mastick & Saltiel 1997) decrease cytoplasmic conductivity as the droplets are electrically insulating and lead to increased opacity.

Apoptosis

Beside many biochemical processes, morphological changes also occur during apoptosis (Cohen 1993; Darzynkiewicz *et al.* 1997; Hengartner 2000). Modifications in the membrane (e.g. membrane blebbing) as well as in the cytoplasm (e.g. cytoplasmic condensation, chromatin aggregation) can change membrane capacitance and cytoplasmic conductivity. With our measurements we could see a separating population at high frequencies (Fig. 5). Because the measurements were performed with unsynchronized cell cultures, data of untreated and treated cultures are broadly scattered. In addition, splitting of CHX-treated population is sprinkled by various data points, indicating transitional stages from normal to apoptotic cells after induction. These apoptosis

studies have also been performed using the Daudi cell line, leading to comparable results (data not shown). With this first analysis we can assume that discrimination of normal from dying cells is possible, and we will add further tests to monitor, beside apoptosis, other cellular processes, such as vitality or activation of transport proteins.

Yeast cell growth and division

By studying cell division of *Saccharomyces*, we could show that impedance measurement can also visualize cellular processes other than apoptosis. During the phases of mitosis, great changes in cell structure occur, such as chromosome condensation and DNA replication, spindle formation and vesiculation of membrane compartments. Particularly in *Saccharomyces* segregation and duplication of many cell constituents occurs in parallel (Pringle *et al.* 1997). Moreover, with yeast cells, we choose a cell model that is different from the usually used animal or human cells. In this context, other microbiological samples were examined in our laboratory, and the results indicate (data not shown) that discrimination of bacteria, bacterial spores and fungi in mixed samples is also possible.

Most promising applications for an impedance spectroscopy flow cytometer are cell differentiation and viability studies, but also many microbiological analyses could benefit from this technology; preliminary experiments on bovine leucocytes infected with parasites (*Theileria*) also show promising results. Whether toxicity studies, another quite interesting application field, could be approached by the described technology depends on the availability of suitable cell models and would demand a feasible adaptation to higher throughput and online capabilities. Finally, for all these applications in which no specific cell markers are known or where fluorescent labels fail, impedance spectroscopy could offer an important option for researchers. That no labels are required, not only significantly reduces costs of assays, but also circumvents the need of frequent and time-consuming sample preparation procedures. This lab-on-a-chip-based device might at present not replace state-of-the-art FACS, but can assist in many cell analyses performed with these rather complex instruments. With the additional improvement of sensor sensitivity, overall performance (event rate), and integration of a cell-sorting capability, impedance spectroscopy flow cytometry would be able to definitely gain access to any analytical cell laboratory.

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REFERENCES

- Arpinati M, Chirumbolo G, Urbini B, Martelli V, Stanzani M, Falcioni S, Bonifazi F, Bandini G, Tura S, Baccarani M, Rondelli D (2000) Use of anti-BDCA-2 antibody for detection of dendritic cells type-2 (DC2) in allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant.* **29**, 887–891.
- Cheung K, Gawad S, Renaud P (2005) Impedance spectroscopy flow cytometry: on-chip label-free cell differentiation. *Cytometry A* **65**, 124–132.
- Cohen JJ (1993) Apoptosis. *Immunol. Today* **14**, 126–130.
- Coulter Electronics (1988) *Method and Reagents for Differential Determination of Four Populations of Leukocytes in Blood*. US patent 4.751.179.
- Cravens PD, Lipsky PE (2002) Dendritic cells, chemokine receptors and autoimmune inflammatory diseases. *Immunol. Cell Biol.* **80**, 497–505.

- Darzynkiewicz Z, Juan G, Li X, Gorczyca W, Murakami T, Traganos F (1997) Cytometry in cell necrobiology: analysis of apoptosis and accidental cell death (necrosis). *Cytometry* **27**, 1–20.
- Fan JY, Carpentier JL, van Obberghen E, Grunfeld C, Gorden P, Orci L (1983) Morphological changes of the 3T3-L1 fibroblast plasma membrane upon differentiation to the adipocyte form. *J. Cell Sci.* **61**, 219–230.
- Fricke H (1924) A mathematical treatment of the electric conductivity and capacity of disperse systems I. The electric conductivity of a suspension of homogeneous spheroids. *Phys. Rev.* **24**, 575–587.
- Gottlieb RA, Nordberg J, Skowronski E, Babior BM (1996) Apoptosis induced in Jurkat cells by several agents is preceded by intracellular acidification. *Proc. Natl Acad. Sci. USA* **93**, 654–658.
- Green H, Meuth M (1974) An established pre-adipose cell line and its differentiation in culture. *Cell* **3**, 127–133.
- Hart DN (1997) Dendritic cells: unique leukocyte populations which control the primary immune response. *Blood* **90**, 3245–3287.
- Hengartner MO (2000) The biochemistry of apoptosis. *Nature* **407**, 770–776.
- Hoffman RA, Britt WB (1978) Flow-system measurement of cell impedance properties. *J. Histochem. Cytochem.* **27**, 234–240.
- Hoffman RA, Johnson TS, Britt WB (1981) Flow cytometric electronic direct current volume and radiofrequency impedance measurements of single cells and particles. *Cytometry* **1**, 377–384.
- Kiesel M, Reuss R, Endter J, Zimmermann D, Zimmermann H, Shirakashi R, Bamberg E, Zimmermann U, Sukhorukov VL (2006) Swelling-activated pathways in human T-Lymphocytes studied by cell volume try and electrorotation. *Biophys. J.* **90**, 4720–4729.
- Liu Y-J, Blom B (2000) Introduction: TH2-inducing DC2 for immunotherapy. *Blood* **95**, 2482–2483.
- Mastick CC, Saltiel AR (1997) Insulin-stimulated tyrosine phosphorylation of caveolin is specific for the differentiated adipocyte phenotype in 3T3-L1 cells. *J. Biol. Chem.* **272**, 20706–20714.
- Pauly H, Schwan HP (1959) Impedance einer suspension von kugelförmigen teilchen mit einer schale. *Zeitung Naturforschung* **14b**, 125–131.
- Pethig R, Kell DB (1987) The passive electrical properties of biological systems: their significance in physiology, biophysics and biotechnology. *Phys. Med. Biol.* **32**, 933–970.
- Pringle JR, Broach JR, Jones EW (1997) *The Molecular and Cellular Biology of the Yeast Saccharomyces*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Rubin CS, Lai E, Rosen OM (1977) Acquisition of increased hormone sensitivity during in vitro adipocyte development. *J. Biol. Chem.* **252**, 3554–3557.
- Santiago-Schwarz F, Anand P, Liu S, Carsons SE (2001) Dendritic cells (DCs) in rheumatoid arthritis (RA): progenitor cells and soluble factors contained in RA synovial fluid yield a subset of myeloid DCs that preferentially activate Th1 inflammatory-type responses. *J. Immunol.* **167**, 1758–1768.
- Schanne OF, P-Ceretti ER (1978) *Impedance Measurements in Biological Cells*. New York: John Wiley & Sohns.
- Schwan HP (1957) Electrical properties of tissue and cell suspensions. *Adv. Biol. Med. Phys.* **5**, 147–209.
- Smith PJ, Wise LS, Berkowitz R, Wan C, Rubin CS (1986) Insulin-like growth factor-I is an essential regulator of the differentiation of 3T3-L1 adipocytes. *J. Biol. Chem.* **263**, 9402–9408.
- Steinman RM, Cohn ZA (1973) Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J. Exp. Med.* **137**, 1142–1162.
- Steinman RM, Witmer MD, Nussenzweig MC, Chen LL, Schlesinger S, Cohn ZA (1980) Dendritic cells of the mouse: identification and characterization. *J. Invest. Dermatol.* **75**, 14–16.
- Tang D, Lahti JM, Grenet J, Kidd VJ (1999) Cycloheximide-induced T-cell death is mediated by a Fas-associated death domain-dependent mechanism. *J. Biol. Chem.* **274**, 7245–7252.
- Wiese RJ, Mastick CC, Lazar DF, Saltiel AR (1995) Activation of mitogen-activated protein kinase and phosphatidylinositol 3'-kinase is not sufficient for the hormonal stimulation of glucose uptake, lipogenesis, or glycogen synthesis in 3T3-L1 adipocytes. *J. Biol. Chem.* **270**, 3442–3446.
- Yang J, Huang Y, Wang X, Wang XB, Becker FF, Gascoyne PRC (1999) Dielectric properties of human leukocyte subpopulations determined by electrorotation as a cell separation criterion. *Biophys. J.* **76**, 3307–3314.