Transient Transfection of the Human Myeloid Cell Line Mono Mac 6 Using Electroporation

BioTechniques 34:142-147 (January 2003)

Niko Klan and Dieter Steinhilber

University of Frankfurt,
Frankfurt, Germany

ABSTRACT

Leukemic cell lines such as Mono Mac 6 provide an excellent model for studying changes in gene expression during induction of cell differentiation. Mono Mac 6 cells can be induced to differentiate from their immature state to cells resembling morphologically and functionally mature monocytes and macrophages by various stimuli such as calcitriol and transforming growth factor-beta. During differentiation, the expression of differentiation markers such as the cell surface antigen CD14 or other differentiation-related genes such as 5-lipoxygenase are strongly increased. Thus, this cell line constitutes an excellent model system to study the regulation of gene expression by inducers of cell differentiation. However, myeloid cell lines are often refractory to transfection by calcium phosphate or DEAE dextran so that reporter gene assays are difficult to perform. We have established a transient transfection protocol for Mono Mac 6 cells using electroporation, a 5-lipoxygenase promoter luciferase reporter gene construct, and the secreted alkaline phosphatase as an internal standard.

INTRODUCTION

Studies of the control of gene expression in eukaryotes strongly rely on the transfer of cloned genes into mammalian cells. The traditional method of gene transfer by the uptake of calcium phosphate/DNA co-precipitates and lipofection works well with fibroblasts and HeLa cells but is much less successful with myeloid cells and myeloid/monocytic cell lines (19,20). Electroporation is a frequently used method for introducing macromolecules and cloned genes into cells that has substantial benefits compared to alternative techniques (30,31). However, transfection efficiency can vary substantially between various cell types. In general, the larger the diameter of the cell, the lower the voltage and capacitance that will be tolerated by the cells before they suffer unacceptable levels of cell death (general killing rates are between 40% and 90%). Increasing the number of cells and the amount of DNA used in the electroporation for studying transient gene expression can circumvent the problems of low transfection efficiency and low promoter/enhancer efficiency. Optimization parameters such as voltage, capacitance, pulse time, composition, and resistance of electroporation media, state of cell growth before electroporation, concentration of DNA, temperature, cell density, and time of assay after transfection (19) are important to obtain acceptable transfection efficiencies. Using the lu-

ciferase reporter gene driven by the 5-lipoxygenase core promoter, we have established a transient transfection system that can easily be used to study gene regulation in the Mono Mac 6 cell line. The 5-lipoxygenase gene expression is upregulated during myeloid cell differentiation (2,3). Characterization of the human 5-lipoxygenase promoter revealed that a part of the promoter sequence (from -114 to +9 from the transcriptional start site) is essential for the transcription of 5-lipoxygenase reporter gene constructs in both HeLa and HL-60 cells (13). Several features of the promoter region such as the lack of TATAA or CCAAT boxes and repeated G+C-rich elements are similar to housekeeping genes. Previous data suggest that the transcription factors Egr-1 and/or Sp1 are required for basal 5-lipoxygenase transcription (14,27) and that they interact with the 5-lipoxygenase promoter and activate it via repeated response elements located between positions -147 and -23 relative to the transcriptional start site (13,22,23). Interestingly, naturally occurring mutations were found in the 5-lipoxygenase promoter, consisting of the deletion of one or two Sp1-binding sites or the addition of one Sp1-binding site (14). These mutations only slightly alter 5-lipoxygenase promoter activity in reporter gene assays but have a significant impact on the response of asthma patients to 5-lipoxygenase inhibitors (7). Recent data obtained with human myeloid cell lines indicate that the 5-

lipoxygenase promoter is regulated by the DNA methylation of the G+C-rich core promoter region (24,25). To study the mechanisms of 5-lipoxygenase promoter regulation, it was important to establish a transient transfection protocol for the 5-lipoxygenase-positive Mono Mac 6 cells.

MATERIALS AND METHODS

Reagents

Molecular biology reagents were from MBI Fermentas, Sigma, Invitrogen, Promega, or other sources as indicated in the text. Insulin was a gift from Aventis (Frankfurt, Germany). Nucleospin[®] Extract columns for direct purification of plasmid DNA (pDNA) were from Macherey-Nagel (Düren, Germany).

Cell Culture

Mono Mac 6 cells were kindly provided by Dr. H.W.L. Ziegler-Heitbrock (Munich, Germany) (28,29). Cells were grown at 37°C in a humidified atmosphere with 6% CO₂ in RPMI 1640 medium supplemented with 10% (v/v) FCS, streptomycin (100 µg/mL), and penicillin (100 U/mL), 1× nonessential amino acids, sodium pyruvate (1 mM), oxalacetate (1 mM), and insulin (10 µg/mL).

Plasmid Constructs

Vector pN10 was obtained by the insertion of the 5-lipoxygenase promoter fragment from -778 to +53 relative to the transcription start site into the promoterless luciferase reporter vector pGL3Basic (Promega, Mannheim, Germany). Plasmid constructs were checked by DNA sequencing.

Transfections

Forty-eight hours before transfection, Mono Mac 6 cells were split to 2 × 10⁵ cells/mL in RPMI 1640 supplemented with 10% FCS, streptomycin (100 µg/mL), penicillin (100 U/mL), 1× nonessential amino acids, sodium pyruvate (1 mM), oxalacetate (1 mM), and insulin (10 µg/mL), and the cells were cultured at 37°C in a humidified

atmosphere with 6% CO₂. For transfection, the cells were harvested by centrifugation at 1200× *g* for 5 min at room temperature and washed twice at room temperature with RPMI 1640 that contained neither FCS nor L-glutamine. The cells were then resuspended at a density of 46 × 10⁶ cells/mL in RPMI 1640, and the cell suspension (0.3 mL) was placed into a 0.4-cm electroporation cuvette. Forty micrograms of supercoiled pDNA were dissolved in water to a final volume of 30 µL and added to the cell suspension in the cuvette. The cells and plasmid were incubated for 5 min at room temperature before electroporation at 975 µF and 200 V (Bio-rad[®] Gene Pulser II) or 200-800 V (Multiporator[®], Eppendorf, Hamburg, Germany), both of which use exponential decay pulse systems. Immediately after the pulse, the cuvettes were transferred into an ice bath for 20 min. The cells were then transferred to 10 mL RPMI 1640 containing 10% FCS, insulin, glutamine, and MEM. Six hours after transfection, the cells were washed once in PBS, pH 7.4, 0.5 mM MgCl₂, 0.5 mM CaCl₂, and lysed in 100 µL lysis buffer (Constant Light Signal kit; Roche Applied Science, Mannheim, Germany).

Reporter Gene Assays

The lysates were assayed for luciferase activity by measuring the light emission in a Microlumat Plus LB96V EG&G Berthold Luminometer using the Constant Light Signal kit. The luminometer was programmed to integrate light emission for 5 s. pCMVSEAP was used as internal standard, and luciferase activity was normalized to secreted alkaline phosphatase (SEAP) activity.

SEAP Activity Assay

SEAP activity was determined with the Phospha-Light[™] Secreted Alkaline Phosphatase Reporter Gene Assay System kit (Tropix, Bedford, MA, USA), and assays were performed according to the manufacturer's instructions using 33 µL conditioned medium of the transfected cells and 100 µL dilution buffer (SEAP kit). Signals were detected with the luminometer that had been pro-

grammed to integrate light emission for 1 s. Generally, SEAP activity was determined 6 h after transfection.

Trypan Blue Stain

The survival of harvested cells was determined by Trypan Blue exclusion. Forty microliters of Trypan Blue (0.4%; Sigma Chemical, Deisenhofen, Germany) were added to 40 µL cell suspension, and the cells were counted after incubation for 5 min at room temperature.

Calculation

Transcriptional activity of pN10 over pGL3Basic (see Figure 3A) was calculated by first subtracting the background mean light unit of mock-transfected cells from the average light unit obtained with the reporter gene luciferase construct for both luciferase and SEAP values, respectively. The corrected light units were then divided by the mean of the light units obtained from pGL3Basic-transfected cells to determine the relative luciferase activity over pGL3Basic as a promoterless vector (16).

When the 5-lipoxygenase promoter activity was determined, the luciferase values were corrected for differences in transfection efficiency by normalizing the luciferase values to the respective value for SEAP, thereby obtaining the relative light units. SEAP values (see Figure 3B) are calculated by subtracting the background mean light unit of mock-transfected cells from the average light unit obtained with the pCMVSEAP construct.

RESULTS

The Field Strength

The field strength (V/cm) of the electrical pulse is an important factor for the cellular survival and transfection efficiency of the cells. If the field strength of the pulse exceeds a characteristic cell-type-specific value, then reversible permeation of the cell membrane occurs. The required voltage depends on the cell diameter and temperature at which electroporation is performed. For Mono Mac 6 cells, which have a diameter of approximate-

Research Report

ly 30 μm , a theoretically expected critical field strength of 444 V/cm can be calculated, which corresponds to a minimum pulse voltage of 180 V if one uses a 0.4-cm cuvette at room temperature. In addition to these parameters, the end volume of the cell suspension in the cuvette must be taken into account. Increasing the pulse voltage can increase the transfection rate but, at the same time, usually also enhances cell mortality. Frequently, killing rates of approximately 50% are observed under experimental conditions that lead to high transfection efficiencies (1,4,6). Figure 1 shows that a stepwise increase in voltage leads to a decrease in cell survival, which indicates a clear relationship between both parameters. When 40 μg 5LO-promoter luciferase reporter gene, 40 μg promoterless vector pDNA (pGL3Basic), and 1 μg pDNA of the pCMVSEAP construct as a control were transfected at 975 μF and the indicated voltages from 50 to 350 V (Figure 2), reporter gene activity was maximal at 200 V, which is slightly higher than the calculated minimum pulse voltage of 180 V. Figure 2 shows that differences of 50 V can decrease reporter gene activity to under 10% of the maximum expression values. These results demonstrate the importance of the optimization of transfection conditions with respect to the transfected cell type (12,15,21).

Thus, using the reporter gene con-

structs with the luciferase reporter gene driven by the human 5-lipoxygenase promoter and the secreted alkaline phosphatase reporter gene driven by the CMV promoter, the voltage of 200 was optimal. When voltages higher than 200 were used, reduced reporter gene activities were obtained.

All transfections were performed at room temperature because the survival of the cells was markedly decreased if electroporations were done at 0°C or if pre-chilled media were used (data not shown and Reference 17).

Length of the Field Pulse

In addition to the field strength, a crucial factor for successful transfection is the pulse length (26). The length of the pulse required for efficient transfection is primarily dependent on the diameter of the cell. The larger the cell, the longer the pulse that is necessary for the permeation of the membrane. Obviously, for the electroporation of Mono Mac 6 cells, a pulse length (time constant) of more than 400 μs seems to be required because when Mono Mac 6 cells were electroporated with the multiporator using pulse lengths 40–400 μs and a cytomegalovirus (CMV) promoter-driven luciferase reporter gene construct, higher voltages of up to 800 V were necessary to obtain sufficient luciferase activity. These luciferase activities were still 80-fold less than the reporter gene activ-

ities obtained with 200 V and 975 μF using the Gene Pulser II, which leads to time constants from $\tau = 33\text{--}39\text{ ms}$. τ describes the decay time for the pulse required to decrease to V_0/e (i.e., about 37% of V_0) (11). After the transfection of the Mono Mac 6 cells according to our protocol, luciferase activities of the CMV promoter-driven reporter gene construct were up to 100-fold higher than the values of the 5-lipoxygenase promoter luciferase reporter gene construct, indicating that the CMV promoter is a much more active promoter than that of 5-lipoxygenase.

Influence of pDNA

The transfection efficiency of electroporation can be affected by the concentration and purity of the pDNA. Therefore, transfections were performed with pDNA purified with Nucleospin Extract columns, and the purity was checked by UV spectroscopy ($A_{260/280} \geq 1.8$) (8,10). Mono Mac 6 cells were transfected with 4, 20, 40, and 80 μg 5-lipoxygenase promoter luciferase plasmid at 200 V and 975 μF , and the luciferase activity was determined 6 h after transfection (Figure 3). Luciferase activity increased with the amount of the transfected plasmid up to 40 μg , resulting in approximately 100-fold higher reporter gene activity compared to the promoterless vector (pGL3Basic) (Figure 3A). SEAP activ-

Figure 1. Mean survival of Mono Mac 6 cells after electroporation. Cells were transfected at the indicated voltages, and the survival of the cells was determined by Trypan Blue exclusion 6 h after electroporation. Transfections were performed in triplicate.

Figure 2. Relation between voltage and reporter gene activities of transiently transfected Mono Mac 6 cells using electroporation. Forty micrograms of the 5-lipoxygenase promoter luciferase reporter gene construct pN10 and 1 μg CMV promoter-driven SEAP reporter gene were transfected at the indicated voltages. Six hours after electroporation, the cells were harvested, and reporter gene assays were performed. Results are indicated as the mean value of the corrected light units and the SEM. LU, light units. Transfections were performed in triplicate.

ity was highest at 20 μ g N10 plasmid DNA and decreased when higher amounts of N10 pDNA (40 or 80 μ g) were applied (Figure 3B).

Influence of the Cell Density

All the results shown were obtained with exponentially growing cells. When cells were overgrown, the transfection efficiency and cell survival decreased significantly (data not shown). Electroporation was performed at a constant volume of 300 μ L per cuvette in all experiments. Under the described electroporation conditions (200 V and 975 μ F), an increase in volume led to shorter pulse times and less cell death but decreased transfection efficiency.

Time Course of Luciferase Activity

Cell recovery after transfection, the rate of transcription from a given promoter, and mRNA stability may influence the time course of reporter gene expression in a given transfection system (19). To obtain maximal reporter gene activity, the time course of luciferase activity must be determined for each cell type (1,17). To optimize the reporter gene assay, 40 μ g pN10 5-lipoxygenase luciferase plasmid were transfected into Mono Mac 6 cells by electroporation at 200 V and 975 μ F, and the luciferase activity was measured at the indicated times following transfection (Figure 4). The luciferase activity peaked 6 h after transfection.

After 21 h, the activity decreased to 8% of the value observed at 6 h, but it was still 20-fold above background.

DISCUSSION

The electroporation techniques are based on the observation that membranes subjected to a high-voltage electric field form pores that are large enough to allow macromol-

ecules to enter or leave the cell (18,31). The pore number and diameter increase with the product of the electric field and pulse duration. Because transfection efficiency sharply peaked at 200 V in Mono Mac 6 cells and almost no transfection occurred at 150 V or lower, it seems that a minimal voltage is required to produce pores, which is in accordance with the calculated critical field strength and the resulting minimal voltage of 180 V under our transfection conditions. Additional increases in the voltage induce pore diameters and total pore areas that are too large for the cell to recover from the transfection, which causes the cells to be irreversibly damaged (Figures 1 and 2). Thus, electroporations of Mono Mac 6 cells should be performed at the optimum voltage (200 V) according to our protocol.

In addition, an important parameter for cell death appears to be the pH change that occurs due to electrolysis close to the electrodes. Some protocols recommend alleviating this problem by reducing the pulse length or a part of the ionic strength of the media or PBS with extra buffer such as HEPES or Tris at pH 7.5, whereas empirical studies and our experience (data not shown) have shown that EDTA and buffer salts such as HEPES or Tris can drastically reduce transfection efficiency (1). When we used short pulses of 40–400 μ s, which lead to low local pH changes and only small amounts of Al^{3+} ions that are solubilized from the electrodes

Figure 3. Effect of DNA concentration on reporter gene activity in Mono Mac 6 cells. The indicated amounts of 5-lipoxygenase promoter construct and 1 μ g pCMVSEAP construct were transfected into Mono Mac 6 cells. Six hours after electroporation, luciferase reporter gene assays (A) and SEAP reporter gene assays (B) were performed, and the results are shown as corrected light units over pGL3Basic vector for the luciferase construct and as corrected light units for SEAP activity. Results are indicated as the mean value of the corrected light units and the SEM of a representative experiment. LU, light units. Transfections were performed in triplicate.

Figure 4. Time course of luciferase activity in Mono Mac 6 cells. Forty micrograms of pN10 and 1 μ g pCMVSEAP (internal standard) were transfected into Mono Mac 6 cells, and the luciferase activity was determined at the indicated times after transfection. Results are given as the relative light units (SEM of at least three experiments). RLU, relative light units.

Research Report

upon pulsing (9), relatively high survival rates were obtained (up to 80% with 975 μ F, 40 μ s, and 200 V); however, luciferase activities were similar to mock-transfected cells, indicating a very low transfection efficiency. The data suggest that the pulse length should be in the range of 30–40 ms.

In addition to the conventional single pulse systems, double pulse systems have been recently developed, based on the rationale that the first high-voltage pulse creates pores or openings in the cell membrane because of the high voltage delivered, and the second low-voltage pulse delivers energy to those cells shocked by the first pulse, therefore favoring the entry of exogenous molecules into the cells by creating an active electrophoresis field. While the transient formation of pores in the plasma membrane following the delivery of the appropriate electrical fields has been documented via elec-

tron microscopy (5), the mechanism of entry of materials through these pores is not known, and no evidence provides for the postulated mechanism. Experimentally, no benefits were observed in our experiments using the double pulse technique (data not shown), which is in agreement with results from other studies (15).

Finally, in the case of pN10, a vector to standard quotient of 40:1 may be regarded as safe because the obtained SEAP activities are still in the dynamic range. Nevertheless, in co-transfection studies, the influence of additional expression vectors on the standard plasmid has to be controlled and, if possible, corrected by keeping the total amount of transfected pDNA constant by the addition of mock pDNA. Care should be taken when choosing the mock pDNA, and individual interactions with the internal standard must be excluded.

Because Mono Mac 6 cells grow

rapidly in suspension and are extremely easy to culture, they represent an interesting cell system to study the regulation of many myeloid genes. In the case of the 5-lipoxygenase promoter, we obtained reporter gene activities in Mono Mac 6 cells that were approximately 1200-fold higher than the promoterless control vector pGL3Basic, whereas much lower promoter activity was found in the 5-lipoxygenase-negative cell line HeLa (33-fold). On the other hand, the 5-lipoxygenase promoter was approximately 100-fold less active than the CMV promoter that was used as the positive control.

Our optimized transfection protocol for Mono Mac 6 cells and the sensitivity of the luciferase assay allows for the characterization of promoters with low and cell-specific transcriptional activity, which eliminates the need for stable transfections that are more time consuming and labor intensive.

ACKNOWLEDGMENTS

We thank Dagmar Szellas and Astrid Brüggerhoff for expert technical assistance. This study was supported by grants from the European Union (QLG1-CT-2001-01521), the Wilhelm Sander Foundation, and the Fonds der Chemischen Industrie.

REFERENCES

- Baum, C., P. Forster, S. Hegewisch-Becker, and K. Harbers. 1994. An optimized electroporation protocol applicable to a wide range of cell lines. *BioTechniques* 17:1058-1062.
- Brungs, M., O. Rådmark, B. Samuelsson, and D. Steinhilber. 1994. On the induction of 5-lipoxygenase expression and activity in HL-60 cells: effects of vitamin D3, retinoic acid, DMSO and TGF β . *Biochem. Biophys. Res. Commun.* 205:1572-1580.
- Brungs, M., O. Rådmark, B. Samuelsson, and D. Steinhilber. 1995. Sequential induction of 5-lipoxygenase gene expression and activity in Mono Mac 6 cells by transforming growth factor β and 1,25-dihydroxyvitamin D3. *Proc. Natl. Acad. Sci. USA* 92:107-111.
- Chang, D.C. 1997. Experimental strategies in efficient transfection of mammalian cells. *Electroporation. Methods Mol. Biol.* 62:307-318.
- Chang, D.C. and T.S. Reese. 1990. Changes in membrane structure induced by electroporation as revealed by rapid-freezing electron microscopy. *Biophys. J.* 58:1-12.
- Chu, G., H. Hayakawa, and P. Berg. 1987. Electroporation for the efficient transfection of mammalian cells with DNA. *Nucleic Acids Res.* 15:1311-1326.
- Drazen, J.M., C.N. Yandava, L. Dube, N. Sczerback, R. Hippensteel, A. Pillari, E. Israel, N. Schork, et al. 1999. Pharmacogenetic association between ALOX5 promoter genotype and the response to anti-asthma treatment. *Nat. Genet.* 22:168-170.
- Ehlert, F., P. Bierbaum, and J. Schorr. 1993. Importance of DNA quality for transfection efficiency. *BioTechniques* 14:546.
- Friedrich, U., N. Stachowitz, A. Simm, G. Fuhr, K. Lucas, and U. Zimmermann. 1998. High efficiency electrotransfection with aluminium electrodes using microsecond controlled pulses. *Bioelectrochem. Bioenerg.* 47:103-111.
- Gaunitz, F., M. Papke, and R. Gebhardt. 1996. Transient transfection of primary cultured hepatocytes using CaPO4/DNA precipitation. *BioTechniques* 20:826-832.
- Hama-Inaba, H., T. Nishimoto, M. Ohtsubo, K. Sato, and M. Kasai. 1988. Simple and effective method of electroporation for introduction of plasmid and cosmid DNAs to mammalian cells. *Nucleic Acids Symp. Ser.* 19:149-152.
- Heiser, W.C. 2000. Optimizing electroporation conditions for the transformation of mammalian cells. *Methods Mol. Biol.* 130:117-134.
- Hoshiko, S., O. Rådmark, and B. Samuelsson. 1990. Characterization of the human 5-lipoxygenase gene promoter. *Proc. Natl. Acad. Sci. USA* 87:9073-9077.
- In, K.H., K. Asano, D. Beier, J. Grobholz, P.W. Finn, E.K. Silverman, E.S. Silverman, T. Collins, et al. 1997. Naturally occurring mutations in the human 5-lipoxygenase gene promoter that modify transcription factor binding and reporter gene transcription. *J. Clin. Invest.* 99:1130-1137.
- Knutson, J.C. and D. Yee. 1987. Electroporation: parameters affecting transfer of DNA into mammalian cells. *Anal. Biochem.* 164:44-52.
- Martin, C.S., P.A. Wight, A. Dobretsova, and I. Bronstein. 1996. Dual luminescence-based reporter gene assay for luciferase and β -galactosidase. *BioTechniques* 21:520-524.
- Maxwell, I.H. and F. Maxwell. 1988. Electroporation of mammalian cells with a firefly luciferase expression plasmid: kinetics of transient expression differ markedly among cell types. *DNA* 7:557-562.
- Neumann, E., M. Schaefer-Ridder, Y. Wang, and P.H. Hofschneider. 1982. Gene transfer into mouse lymphoma cells by electroporation in high electric fields. *EMBO J.* 1:841-845.
- Pahl, H.L., T.C. Burn and D.G. Tenen. 1991. Optimization of transient transfection into human myeloid cell lines using a luciferase reporter gene. *Exp. Hematol.* 19:1038-1041.
- Pahl, H.L., A.G. Rosmarin, and D.G. Tenen. 1992. Characterization of the myeloid-specific CD11b promoter. *Blood* 79:865-870.
- Putirka, S. and M. Iacobelli. 1993. Optimizing electroporation of mammalian cells. *Am. Biotechnol. Lab.* 11:18.
- Silverman, E.S. and J.M. Drazen. 2000. Genetic variations in the 5-lipoxygenase core promoter. Description and functional implications. *Am. J. Respir. Crit. Care Med.* 161:S77-S80.
- Silverman, E.S., J. Du, G.T. De Sanctis, O. Rådmark, B. Samuelsson, J.M. Drazen, and T. Collins. 1998. Egr-1 and Sp1 interact functionally with the 5-lipoxygenase promoter and its naturally occurring mutants. *Am. J. Respir. Cell Mol. Biol.* 19:316-323.
- Singal, R. and G.D. Ginder. 1999. DNA methylation. *Blood* 93:4059-4070.
- Uhl, J., N. Klan, M. Rose, K.D. Entian, O. Werz, and D. Steinhilber. 2002. The 5-lipoxygenase promoter is regulated by DNA methylation. *J. Biol. Chem.* 277:4374-4379.
- Wolf, H., M.P. Rols, E. Boldt, E. Neumann, and J. Teissie. 1994. Control by pulse parameters of electric field-mediated gene transfer in mammalian cells. *Biophys. J.* 66:524-531.
- Zhang, D.E., C.J. Hetherington, S. Tan, S.E. Dziennis, D.A. Gonzalez, H.M. Chen, and D.G. Tenen. 1994. Sp1 is a critical factor for the monocytic specific expression of human CD14. *J. Biol. Chem.* 269:11425-11434.
- Ziegler-Heitbrock, H.W., W. Schraut, P. Wendelgass, M. Strobel, T. Sternsdorf, C. Weber, M. Aepfelbacher, M. Ehlers, et al. 1994. Distinct patterns of differentiation induced in the monocytic cell line Mono Mac 6. *J. Leukoc. Biol.* 55:73-80.
- Ziegler-Heitbrock, H.W., E. Thiel, A. Futterer, V. Herzog, A. Wirtz, and G. Riethmüller. 1988. Establishment of a human cell line (Mono Mac 6) with characteristics of mature monocytes. *Int. J. Cancer* 41:456-461.
- Zimmermann, U. 1996. The effect of high intensity electric field pulses on eukaryotic cell membranes: fundamentals and application, p. 1. In U. Zimmermann and G.A. Neil (Eds.), *Electromanipulation of Cells*. CRC Press, Boca Raton, FL.
- Zimmermann, U., F. Riemann, and G. Pilwat. 1976. Enzyme loading of electrically homogeneous human red blood cell ghosts prepared by dielectric breakdown. *Biochim. Biophys. Acta* 436:460-474.

Received 18 June 2002; accepted 18 September 2002.

Address correspondence to:

Dr. Dieter Steinhilber
Institute of Pharmaceutical Chemistry
University of Frankfurt
Marie-Curie-Str. 9
60439 Frankfurt, Germany
e-mail: steinhilber@em.uni-frankfurt.de

For reprints of this or
any other article, contact
Reprints@BioTechniques.com