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Electroporation in 'intracellular' buffer increases cell survival

Maurice J.B.van den Hoff, Antoon F.M.Moorman and Wouter H.Lamers*
University of Amsterdam, Department of Anatomy and Embryology, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands

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The electroporation method has been shown to be useful technique for introducing DNA into mammalian cells (1). It is believed that cell poration is caused by reversible electrical breakdown of the cell membrane (2). Previously, we have shown that upon electroporation the cells are sensitive to the osmolarity (3) and ionic composition of the medium. A similar conclusion was reached for chemically permeabilized cells which can be successfully maintained in buffers that resemble the intracellular ionic composition (cytomix: 120 mM KCl; 0.15 mM $CaCl_2$; 10 mM K_2HPO_4/KH_2PO_4, pH 7.6; 25 mM Hepes, pH 7.6; 2 mM EGTA, pH 7.6; 5mM $MgCl_2$; pH adjusted with KOH), and used for metabolic studies (4). Shortly before use freshly prepared ATP (2 mM; pH = 7.6 adjusted with KOH) and glutathione (5 mM) were added to prevent leakage of the cytoplasmic components, protect membranes against oxidation and facilitate resealing of pores (5: A.J.Meijer, personal communication). Normally cells are electrotransfected in either culture medium of PBS (150 mM NaCl; 10 mM Na_2HPO_4/NaH_2PO_4, pH 7.4). We hypothesized that cells would survive electrotransfection more readily in cytomix, rather than in culture medium or PBS, as these media contain ions at concentrations that are detrimental to the cell.

Rat hepatoma cells (FTO-2B) were grown to subconfluence, trypsinized and harvested. After washing twice in PBS, 1×10^7 cells were pelleted and resuspended either in culture medium (DMEM/F12; Gibco 074–2400P), PBS or cytomix, each supplemented with 50 μg pRSVCAT and 5 μg pSV2Luc. This suspension was electroshocked in a 2 mm cuvette placed in a Biorad Gene Pulser apparatus with Capacitance Extender under the conditions specified in Table 1. After electroporation the cells were plated and cultured for 48 hours in DMEM/F12, containing 2.5% FCS and 2.5% NCS. Protein concentration, chloramphenicol acetyltransferase (CAT) and luciferase activities (Luc) were determined in cell extracts (6, 7).

At 500 μF efficiency of transfection (reflected by CAT activity) and cell survival and were highest in cytomix, and lowest in PBS (Table 1). By elevating the capacity to 960 μF, the differences between cell viability in the electroporation media became more pronounced, while transfection efficiency (in cytomix) further increased. At 500 or 960 μF, τ (time required for the potential to decay to 1/e of the peak value) was comparable in all media, indicating a similar resistance. Switching two Biorad Capacitance Extenders in parallel, in cytomix the highest CAT activity was obtained at a capacity of 1435 μF (one on 960, the other on 500 μF) at the expense of a 2-fold decrease in cell viability. At the highest capacity setting (1895 μF) CAT activity and cell viability were lower than at 1435 μF. Increasing the voltage to 450V generated too high potential in the cuvette, creating a break-down voltage, and uncontrolled pulse. The similarity of the ratio of CAT and Luc activities under all conditions demonstrates an equal access of both constructs to the cells.

It is concluded that electroporation of cells in cytomix combines a high efficiency of transfection with a good rate of cell survival.

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Table 1. Efficiency of transfection and cell viability under different conditions of electroporation. Viability is expressed as percentage of cells surviving after electroporation. Values are mean ± S.E.M.; n = number of independent transfections; n.d. = not determined (due to poor cell survival).

Conditions	n	CAT mU/μg protein	viability %	CAT/Luc
PBS				
300V, 500 μF	3	0.33 ± 0.01	43 ± 2	0.024 ± 0.003
300V, 960 μF	3	n.d.	4 ± 2	n.d.
DMEM/F12				
300V, 500 μF	6	0.58 ± 0.08	52 ± 8	0.020 ± 0.004
300V, 960 μF	3	n.d.	11 ± 2	n.d.
cytomix				
300V, 500 μF	3	0.66 ± 0.06	69 ± 5	0.013 ± 0.002
300V, 960 μF	6	1.63 ± 0.63	57 ± 4	0.024 ± 0.004
300V, 1435 μF	3	3.94 ± 0.34	29 ± 5	0.021 ± 0.001
300V, 1895 μF	3	1.18 ± 0.20	13 ± 2	0.018 ± 0.002
450V 960 μF	3	1.40 ± 0.14	17 ± 3	0.017 ± 0.003

* To whom correspondence should be addressed