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A semi-automated method for isolating functionally intact mitochondria from cultured cells and tissue biopsies

Sabine Schmitt [a,1], Friederike Saathoff [b,1], Lilja Meissner [c], Eva-Maria Schropp [a], Josef Lichtmannegger [a], Sabine Schulz [a], Carola Eberhagen [a], Sabine Borchard [a], Michaela Aichler [d], Jerzy Adamski [e], Nikolaus Plesnila [c], Simon Rothenfusser [b], Guido Kroemer [f,g,h,i,j], Hans Zischka [a,*]

[a] Institute of Molecular Toxicology and Pharmacology, Helmholtz Center Munich, German Research Center for Environmental Health, D-85764 Neuherberg, Germany
[b] Division of Clinical Pharmacology, Department of Medicine IV, Ludwig Maximilian University Munich, D-80336 Munich, Germany
[c] Institute for Stroke and Dementia Research, University of Munich, Medical Center–Campus Grosshadern, D-81377 Munich, Germany
[d] Research Unit Analytical Pathology–Institute of Pathology, Helmholtz Center Munich, German Research Center for Environmental Health, D-85764 Neuherberg, Germany
[e] Genome Analysis Center–Institute of Experimental Genetics, Helmholtz Center Munich, German Research Center for Environmental Health, D-85764 Neuherberg, Germany
[f] INSERM, U848, 94805 Villejuif, France
[g] Université Paris Descartes/Paris V, Sorbonne Paris Cité, 75270 Paris, France
[h] Metabolomics Platform, Institut Gustave Roussy, 94805 Villejuif, France
[i] Equipe 11 labellisée Ligue contre le Cancer, Centre de Recherche des Cordeliers, 75006 Paris, France
[j] Pôle de Biologie, Hôpital Européen Georges Pompidou, AP–HP, 75908 Paris, France

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ABSTRACT

Mitochondrial dysfunctions decisively contribute to the progression of human diseases, implying that functional tests of isolated mitochondria may furnish conclusive information for diagnosis and therapy. Classical mitochondrial isolation methods, however, lack precisely adjustable settings for cell rupture, which is the most critical step in this procedure, and this complicates subsequent analyses. Here, we present an efficient method to isolate functionally active, intact mitochondria from cultured or primary cells and minute tissue samples in a rapid, highly reproducible manner.

Mitochondria are organelles that control bioenergetics, metabolic signaling, and cell death decisions [1]. Mitochondrial dysfunctions are causative in a plethora of human diseases [2], and so mitochondria constitute a promising therapeutic target [3].

Although mitochondrial functions can be studied in situ, investigating isolated mitochondria offers an unmatched advantage [4]. Indeed, only cell-free systems allow one to demonstrate that a disease-associated functional defect is intrinsic to mitochondria and that pathogenic conditions or pharmacological agents affect these organelles in a direct fashion [5].

The isolation of mitochondria requires an initial step of cell/tissue homogenization that is followed by subsequent steps of purification, usually by differential centrifugation. This general isolation approach was established during the late 1940s [6–8] and has been applied to mitochondria from innumerous species and tissues. However, the protocols differ dramatically with respect to the initial homogenization step, requiring adaptations to distinct cell types or tissues. This step is the most critical one because it needs to be rough enough to efficiently break the plasma membrane but mild enough to prevent mitochondrial damage. One would intuitively expect that this delicate balance is dealt with by precise and finely tunable instruments to ensure reproducibility or at least controllability. However, rather the opposite is the case. Most homogenization protocols rely on the "classical" Teflon/glass–glass potter, nitrogen cavitation, or other mechanical or chemical means that are far from being precisely adjustable, resulting in inter-experiment variability. Moreover, if applied to various cell lines or minute tissue samples, at least in our hands, these procedures usually fail to yield mitochondria that would be comparable in abundance, purity, and integrity to density gradient purified rat liver mitochondria, the "gold standard" in mitochondrial research [9].

Driven by these premises, we employed the "Balch homogenizer," an accurately carved metal apparatus that was designed approximately three decades ago, for high-precision cell breakage [10]. Using a current version (Isobiotec), cell suspensions are passed through a clearance of 4–18 μm, which is precisely

* Corresponding author. Fax: +49 89 3187 3449.
E-mail address: zischka@helmholtz-muenchen.de (H. Zischka).
[1] Both authors share first authorship.

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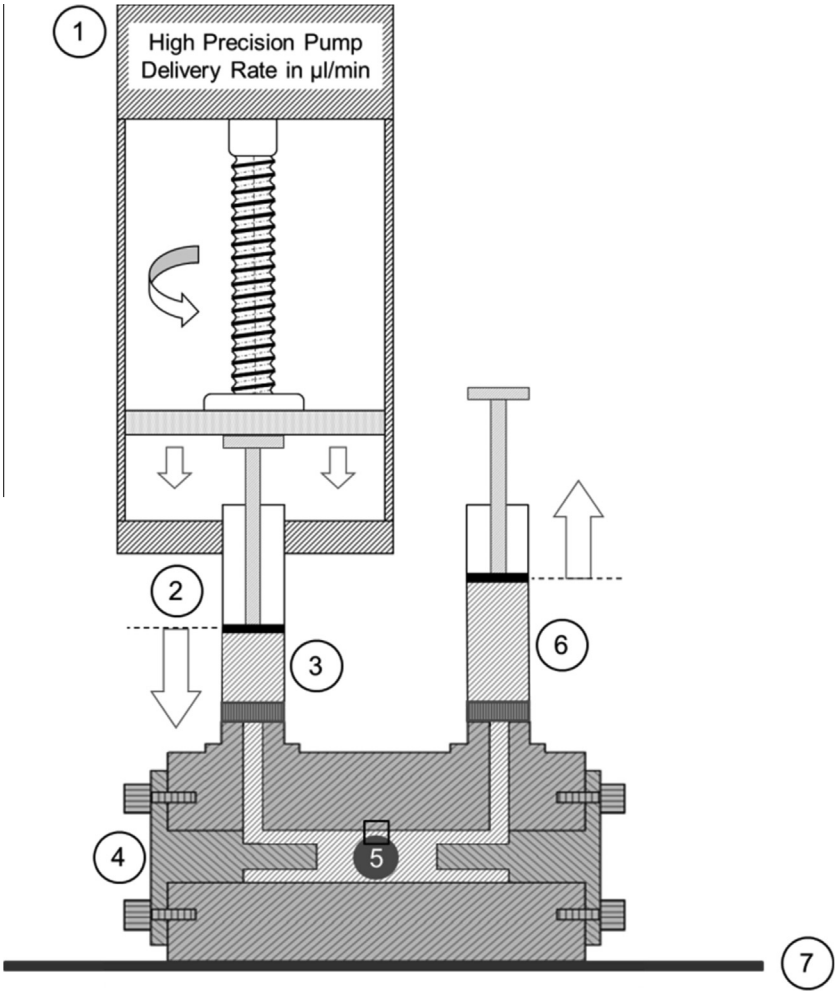

Fig.1. The pump-controlled cell rupture system (PCC). A high-precision pump (1) ensures, via gas-tight syringes (2), the continuous sample delivery (3) in a constant rate to the Balch homogenizer (4). Cell breakage occurs on passage through a defined clearance (square), which is adjusted by selecting tungsten carbide balls of different diameters (5). The cell homogenate is collected in a second syringe (6) and can be resubjected to the homogenizer, which is thermally equilibrated by a cooling plate (7).

determined by spherical tungsten carbide balls of selectable diameters (Fig. 1). Although it has been successfully employed, reports using the Balch homogenizer are scarce [10–16], especially with respect to the isolation of mitochondria [17]. Here, we report a method in which the Balch homogenizer is coupled to a high-precision pump system, resulting in an exactly tunable, operator-independent cell homogenization unit that we term the "pump-controlled cell rupture system" (PCC)[2] (Fig. 1).

Materials and methods

Animals

Rats were obtained from Charles River Laboratories (WKY and Buffalo) or were provided by Jimo Borjigin (University of Michigan) (LPP, homozygous or heterozygous). Mice (CD1 and C57/Bl6) were obtained from the Helmholtz Center Munich. Animals were housed under the guidelines for the care and use of laboratory animals at the Helmholtz Center Munich or at the Institute for Stroke and Dementia Research, University of Munich, Medical Center–Campus Grosshadern.

Cell culture

McA 7777, HEK 293, HeLa, 1205Lu, Panc02, and MEF cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma–Aldrich, Germany) with 1% glutamate (GlutaMAX, Gibco, UK). HepG2 cells were cultured in RPMI 1640 + GlutaMAX. McA 7777 cells were also cultured in glucose-free DMEM supplemented with 10 mM galactose, 2% glutamate (GlutaMAX) and 1 mM sodium pyruvate (PAA Laboratories, Austria). All media were supplemented with 10% fetal calf serum (FCS, Biochrom, Germany) and 1% penicillin/streptomycin (Gibco). The cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2. For mitochondria isolation, cells were trypsinized and separated/singularized with a syringe. Only cell suspensions with more than 80% vitality were used for the isolation of mitochondria.

Primary hepatocytes were isolated as described previously [18]. Briefly, rats were anesthetized with Narcoren (100 mg/kg, Merial, Germany). Perfusion buffers were purged with carbogen and kept at 37 °C for 15 min. The liver was perfused with Hank's balanced

[2] *Abbreviations used:* PCC, pump-controlled cell rupture system; DMEM, Dulbecco's modified Eagle's medium; HBSS, Hank's balanced salt solution; ddH_2O, double distilled water; $\Delta\psi_m$, mitochondrial inner transmembrane potential; ZE–FFE, zone electrophoresis in a free-flow electrophoresis device; BSA, bovine serum albumin; RCR, respiratory control ratio; Rh123, rhodamine 123; ANT, adenine nucleotide translocator; VDAC, voltage-dependent anion channel; Cyt c, cytochrome *c*; AIF, apoptosis-inducing factor; ER, endoplasmic reticulum; MPT, mitochondrial permeability transition; CsA, cyclosporin A; FCCP, carbonylcyanide-ρ-(trifluoromethoxy) phenyl-hydrazone.