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Single-cell lysis for visual analysis by electron microscopy

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ABSTRACT

The stochastic nature of biological systems makes the study of individual cells a necessity in systems biology. Yet, handling and disruption of single cells and the analysis of the relatively low concentrations of their protein components still challenges available techniques. Transmission electron microscopy (TEM) allows for the analysis of proteins at the single-molecule level. Here, we present a system for single-cell lysis under light microscopy observation, followed by rapid uptake of the cell lysate. Eukaryotic cells were grown on conductively coated glass slides and observed by light microscopy. A custom-designed microcapillary electrode was used to target and lyse individual cells with electrical pulses. Nanoliter volumes were subsequently aspirated into the microcapillary and dispensed onto an electron microscopy grid for TEM inspection. We show, that the cell lysis and preparation method conserves protein structures well and is suitable for visual analysis by TEM.

1. Introduction

The aim of systems biology is to understand the emergence of biological functions from interaction networks (Westerhoff, 2011). This requires knowledge of the intracellular players and their interconnections, for which an inventory of the individual components of the system, i.e. the transcriptome, the proteome, the metabolome and, finally, the interactome, has to be assembled. Such an inventory will strongly vary from cell to cell, as the stochastic nature of biological processes leads to "biological noise" (Eldar and Elowitz, 2010; Raj and van Oudenaarden, 2008). This makes the study of individual systems, e.g., single cells, a necessity (Wang and Bodovitz, 2010).

Genome sequencing (Zong et al., 2012) and expression profiling (Flatz et al., 2011) are far advanced, and amplification techniques are ready to be applied to single cells (Kalisky et al., 2011). The analysis of the metabolism of a biological system profits from the experience and advances of analytical chemistry (Fiehn, 2001); for example, mass spectrometry (MS) can be used to identify metabolites with single-cell sensitivity (Amantonico et al., 2008). Moreover, excellent imaging techniques, such as light- and electron microscopy (EM) or X-ray diffraction imaging, are available for structural analyses.

However, proteomic studies at the single-cell level are hampered by the low expression level of many proteins and the lack of amplification techniques. Although powerful and valuable techniques, such as MS (Picotti et al., 2009) and cryo-electron tomography (cryo-ET) (Henderson et al., 2007; Medalia et al., 2002; Nickell et al., 2006), are applied for single-cell proteomic studies, such studies still remain a challenging task, especially for eukaryotic cells (Bantscheff et al., 2007; Diebolder et al., 2012; Mader et al., 2010). Thus, adjuvant techniques utilizing novel or hybrid approaches are beneficial to further untangle the complexity of single-cell protein networks.

A combination of microfluidics and TEM was suggested as an alternative and complementary approach to investigate the protein content of single eukaryotic cells (Engel, 2009, 2010). The idea is to

Abbreviations: AM, ammonium molybdate; BHK, baby hamster kidney; ddH_2O, double-distilled water; ECL, enhanced chemiluminescence; EM, electron microscopy; ET, electron tomography; FEA, finite element analysis; FS, fused silica; HRP, horseradish peroxidase; ID, inner diameter; ITO, indium tin oxide; MS, mass spectrometry; OD, outer diameter; OM, optical microscope; PDMS, poly(dimethylsiloxane); PEEK, poly(ether–ether–ketone); RPPA, reverse-phase protein arrays; SNR, signal-to-noise ratio; TEM, transmission electron microscopy; UA, uranyl acetate.

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physically lyse single cells and spread the entire sample onto EM grids for structural analysis by transmission electron microscopy (TEM), or mass measurements by scanning TEM (STEM). This "lyse and spread" approach provides access to EM imaging at a higher signal-to-noise ratio (SNR) than when in the cellular background, and enables a more straightforward correlation of structural information with mass data. A prerequisite of this envisaged approach is a targeted lysis of individual cells and an efficient preparation of their lysate for TEM analysis.

A variety of different techniques for single-cell lysis exists today, and most of them have been implemented in microfluidic systems (Brown and Audet, 2008). Many of these systems utilize the principle of electroporation (Fox et al., 2006; Movahed and Li, 2011) to lyse detached or suspended cells in flow-through configurations, whereas only a few report on electrical lysis of "standard" adherent eukaryotic cells in cultures (Han et al., 2003; Nashimoto et al., 2007). However, despite their popularity none of these systems has been utilized to prepare samples of a single-cell lysate for electron microscopy.

Here, we present a system for the electrical lysis of individual adherent eukaryotic cells and subsequent preparation of minute sample volumes for negative-stain TEM. The setup includes a custom-designed microcapillary electrode (Fig. 1), which targets and lyses individual cells observed in a light microscope. Immediately after lysis, the cell-fragments are aspirated into the microcapillary, deposited on an EM grid and negatively stained. This method offers the potential for an alternative approach to analyze proteins and protein complexes from individual eukaryotic cells.

2. Materials and methods

2.1. Instrument setup

The principle and basic design of the system developed for the electrical lysis of single cells is shown in Fig. 1 A. The system is designed for use with an inverted optical microscope (OM; Zeiss Axiovert 40C). The microscope is equipped with a custom-built stage that has a customized mounting frame on the objective guide. The latter accommodates an indium tin oxide (ITO)-coated glass slide (ground electrode and sample platform) and can be moved manually in the xy-plane. Miniaturized Petri dishes on the surface of the glass slide (see below) allow cell cultures to be grown. A tapered gold-coated microcapillary that serves as second electrode can be positioned in close proximity above the glass slide. The upper un-tapered end of the microcapillary is inserted in a steel adapter and electrically connected with silver paint (Fig. 1B). The insulating holder of the adapter is attached to a stepper motor (PI M-126.PD2, Physik Instrumente, Germany) mounted on top of an xy-platform on the microscope stage. This platform allows the microcapillary tip to be centered above the objective lens of the OM. The stepper motor moves the capillary in the z-direction. The other end of the steel adapter holding the capillary is connected via a PEEK (poly(ether–ether–ketone)) tube (inner diameter (ID) 250 μm) to a pressure reservoir (Fig. 1). The tube is intercepted by a solenoid valve (LVFA0550310H, The Lee Company, USA) that is controlled by the computer through an NI USB-6009 module. A pressure controller (PCNC-0001-00, Seyonic, Switzerland) is used to apply positive or negative pressure to the system via the pressure reservoir. A function generator (33220A, Agilent, Switzerland) delivers a voltage signal, which is amplified 20 times by a linear voltage amplifier (F20A, FLC Electronic AB, Sweden). The output of the linear amplifier is electrically connected to the capillary, and the conductive glass slide is grounded. All electronic components of the system are controlled by a LabVIEW-based, custom-made software (Supp. Fig. 1). A camera (GC750 GigE, Prosilica, USA), mounted on the microscope, enables live-cell imaging and video recording. The next version of the control software will be available as an open-source plug-in for the openBEB (open biological experiment browser) system (www.openBEB.org).

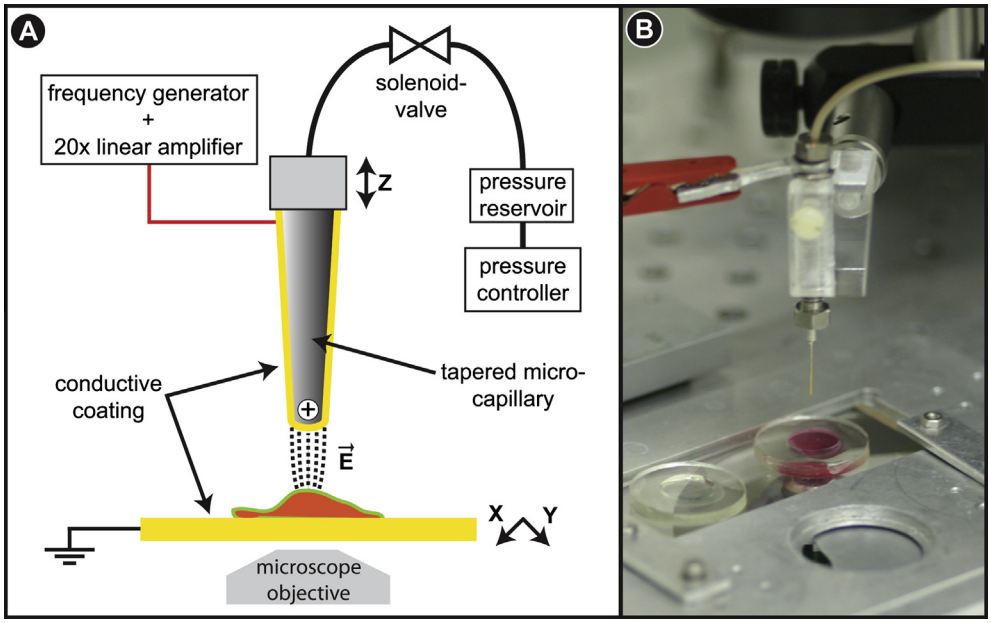

Fig.1. Single-cell lysis instrumentation. (A) Schematic representation of the single-cell lysis setup, which is mounted on an inverted optical microscope. A camera allows for live-cell imaging. The stepper motor approaches the gold-coated microcapillary to the ITO-coated glass slide, where individual cells can be targeted. The function generator sends a voltage pulse to the capillary tip to lyse the cell. Meanwhile, the pressure controller builds up a negative pressure on the closed solenoid valve. Upon cell lysis, the valve is opened for a defined period of time, and the cell lysate is aspirated into the capillary. (B) Lysis microcapillary and cell culture slide. The upper end of the gold-coated microcapillary is inserted into a steel adapter and electrically connected with silver paste. The other end of the steel adapter is attached to a piece of PEEK tubing that connects the microcapillary to the pressure controller via a pressure reservoir. The microcapillary is positioned above a grounded, ITO-coated, glass slide with a PDMS ring on its surface. Slide and ring form a mini Petri dish that can be filled with cell culture medium (red).