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## REVIEW

## Current techniques for single-cell lysis

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Owing to the small quantities of analytes and small volumes involved in single-cell analysis techniques, manipulation strategies must be chosen carefully. The lysis of single cells for downstream chemical analysis in capillaries and lab-on-a-chip devices can be achieved by optical, acoustic, mechanical, electrical or chemical means, each having their respective strengths and weaknesses. Selection of the most appropriate lysis method will depend on the particulars of the downstream cell lysate processing. Ultrafast lysis techniques such as the use of highly focused laser pulses or pulses of high voltage are suitable for applications requiring high temporal resolution. Other factors, such as whether the cells are adherent or in suspension and whether the proteins to be collected are desired to be native or denatured, will determine the suitability of detergent-based lysis methods. Therefore, careful selection of the proper lysis technique is essential for gathering accurate data from single cells.

**Keywords:** cell lysis; single-cell analysis; cell sampling

## 1. INTRODUCTION

Single-cell studies are crucial in order to productively study the complexity of intracellular processes. However, tools that are capable of harvesting large amounts of proteomic data from single cells remain rather limited, largely owing to the difficulty involved in dealing with the small volumes and quantities of analytes concerned. Despite the limitations, over the last decade or two, there has been significant progress in developing assays capable of determining levels of specific proteins (Zhang & Jin 2004, 2006; Huang *et al.* 2007) and interrogating enzyme activity in single cells (Meredith *et al.* 2000; Li *et al.* 2004; Ocvirk *et al.* 2004). One of the most limiting aspects for these studies to date has been the controlled disruption of selected cells. Depending on the downstream analysis to be performed, the cell lysis technique may require specific attributes. Analysis of processes that are highly dynamic such as signal transduction mechanisms will require lysis procedures that are significantly faster than the dynamics of the measured entity. Downstream enzyme assays require extraction of the enzyme from the cell in its active form (not denatured). Additionally, for downstream separation processes, the lysis conditions must

not involve the addition of chemicals that are detrimental to the separation procedure or that alter properties of the analyte. Finally, the ability to integrate the chosen lysis technique into the experimental platform, such as interfacing with capillaries for electrophoretic analysis, or integration into complex microfluidic lab-on-a-chip devices should be considered.

The objective of this review is to illustrate the lysis methods that have been used to date for the analysis of single cells, and to examine the limitations of these techniques. For the purposes of this review, the different approaches used to lyse cells have been grouped into five categories: optical; mechanical; acoustic; electrical; and chemical. As a comparative tool, the details of the cell lysis modes and applications of the studies referred to in the body of this review have been compiled in table 1.

## 2. OPTICAL LYSIS

The application of laser pulses to rapidly lyse individual cells has come about relatively recently. Pulsed laser microbeam-induced cell lysis involves directing a nanosecond pulse from a 532 nm laser through a high numerical aperture objective lens and thus focused down to a small spot where localized plasma formation occurs. This results in the generation of a shock wave, followed by generation of a cavitation bubble that expands and contracts within a matter of microseconds (Rau *et al.* 2004, 2006). The cells located near the centre of the targeted area have been shown to lyse

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One contribution of 7 to a Theme Supplement 'Single-cell analysis'.

Table 1. Comparative list of single-cell lysis studies.

lysis technique	application	cell type	platform	reference
laser pulse 532 nm	subcellular sampling of fluorescent dye/dye labelled peptides	RBL, PC12	capillary electrophoresis	Li <i>et al.</i> (2001a)
laser pulse 532 nm	characterization of lysis and poration of cells	PtK2	high-speed imaging	Hellman <i>et al.</i> (2007)
laser pulse 532 nm	characterization of cell lysis method	PtK2	high-speed imaging	Rau <i>et al.</i> (2004, 2006)
laser pulse 532 nm	characterization of cell lysis method	BAF-3	high-speed imaging in micro-fluidic channel	Quinto-Su <i>et al.</i> (2008)
laser pulse 532 nm	PKA, PKC, CamKII and cdc2K enzyme activity	RBL, Swiss 3T3	capillary electrophoresis	Meredith <i>et al.</i> (2000)
laser pulse 532 nm	PKB enzyme activity	HT1080, NIH 3T3	capillary electrophoresis	Li <i>et al.</i> (2004)
laser pulse 532 nm	PKC, CamKII enzyme activity	RBL	capillary electrophoresis	Li <i>et al.</i> (2001b)
laser pulse 532 nm	characterization and optimization of lysis method	RBL	capillary electrophoresis	Sims <i>et al.</i> (1998)
laser pulse 355 nm	$\beta$ -galactosidase enzyme assay	mast cells	imaging	He <i>et al.</i> (2005)
nanoscale barbs	characterization of lysis efficiency	HL-60, whole blood	microfluidic chip	Di Carlo <i>et al.</i> (2003)
electrical (20 kV cm <sup>-1</sup> 10 $\mu$ s pulse)	mRNA quantification	MCF-7, HMT-3522	PCR	Nashimoto <i>et al.</i> (2007)
electrical (300 V cm <sup>-1</sup> )	characterization of cell lysis method	AML	microfluidic chip electrophoresis	Munce <i>et al.</i> (2004)
electrical (various conditions 10–20 V/10–60 $\mu$ m for 200–1000 $\mu$ s)	characterization and optimization of lysis method	RBL	capillary electrophoresis	Han <i>et al.</i> (2003)
electrical (Tesla coil)	LDH enzyme activity	lymphocytes	capillary electrophoresis	Xue & Yeung (1996)
electrical/hypotonic (600 V cm <sup>-1</sup> 1 s pulse)	GSH quantification	erythrocytes	microfluidic chip electrophoresis	Gao <i>et al.</i> (2004)
electrical/surfactant (450–900 V cm <sup>-1</sup> (DC+AC) 3% pluronic P84)	characterization of serial cell lyses	Jurkat	microfluidic chip electrophoresis	McClain <i>et al.</i> (2003)
sonication/detergent (digitonin)	interferon- $\gamma$ isoform quantification	NK	capillary electrophoresis	Zhang & Jin (2004, 2006)
detergent (0.1% Triton X-100)	optimization of lysis method	MDCK	capillary electrophoresis	Berezovski <i>et al.</i> (2007)
detergent (0.1% Triton X-100)	$\alpha$ -glucosidase I and II, 1,3- <i>N</i> -acetylgalactos-aminyltransferase and $\beta$ -galactosidase enzyme activity	Sf9, HT29, <i>Arabidopsis</i> embryos	capillary electrophoresis	Shoemaker <i>et al.</i> (2005)
detergent/hypotonic (5 mM SDS)	PKA enzyme activity	C2C12	capillary electrophoresis	Zarrine-Afsar & Krylov (2003)
detergent (multiple: anionic, zwitterionic, non-ionic)	protein–DNA complexes	MDCK	capillary electrophoresis	Pang <i>et al.</i> (2006)
chemical/detergent (3 M GTC, 0.2% SDS or 0.1% SDS)	quantification of insoluble actin	MOLT-3	microfluidic chip	Irimia <i>et al.</i> (2004)
detergent (1% DDM)	$\beta_2$ -adrenergic receptor and phycobiliprotein quantification	Sf9 and PCC 7942	microfluidic chip electrophoresis	Huang <i>et al.</i> (2007)
detergent/hypotonic (25 mM SDS)	farnesyltransferase, endoprotease and methyltransferase activity	4T1 and NIH 3T3	capillary electrophoresis	Arkhipov <i>et al.</i> (2005)
detergent/hypotonic (20 mM SDS)	development and optimization of serial adherent cell lyses	RBL	microfluidic chip-fed capillary electrophoresis	Marc <i>et al.</i> (2007)
chemical (20 mM OH <sup>-</sup> generated at cathode)	characterization and optimization of lysis method	erythrocytes, HeLa, CHO	microfluidic chip	Di Carlo <i>et al.</i> (2005)

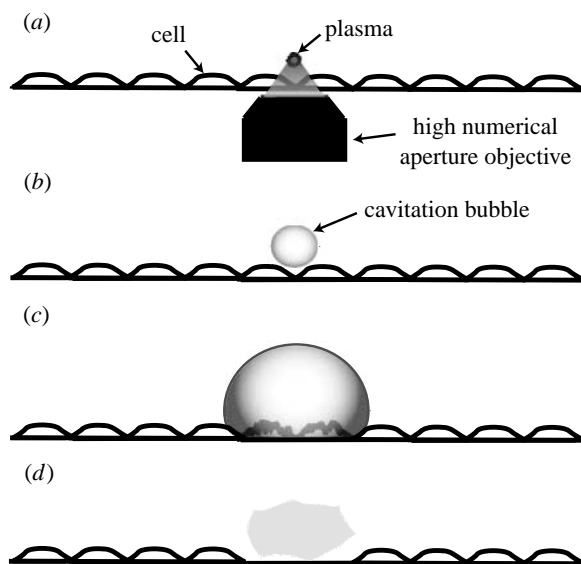


Figure 1. Schematic of the progressive stages involved in the laser-induced cell lysis. (a) A laser micropulse is focused in proximity to a targeted cell, (b) cavitation bubble forms centred around the focused laser pulse, (c) the expanding cavitation bubble disrupts cells and (d) the cells within the zone of injury are selectively lysed.

either during the expansion of the cavitation bubble (less than  $1\ \mu\text{s}$  after pulse) when the focal point of the laser pulse is directed near the cells (approx.  $10\ \mu\text{m}$  above), as shown in figure 1, or during bubble collapse, when a liquid jet is directed downwards onto the slide (approx.  $30\ \mu\text{s}$  after pulse) when it is focused significantly higher (approx.  $400\ \mu\text{m}$ ; Rau *et al.* 2006). When applied to sheets of adherent cells, the cells located near the centre of the pulse were completely disrupted, the cells further away remained adherent but were found to be necrotic, and the remaining adherent cells around the lysis region remain viable but become porated as evidenced by the uptake of 3 kDa FITC-dextran. The distances from the laser focal point at which these three outcomes occurred were related to shear stress levels, showing larger lysis radii with increasing pulse energies (Hellman *et al.* 2007). Owing to the large irradiances used to achieve cell lysis and the subsequent creation of plasma in this process, it is important to ensure that the cell contents are not being fundamentally altered by these processes. In an attempt to address this issue, the sampling efficiency of this technique has been examined using green fluorescent protein (GFP)-transfected cells (Brown & Audet 2007). In this study, however, the laser pulse was directed below the cell near the buffer–glass slide interface, resulting in the disappearance of the cell from one frame of video (sampled at 30 Hz) to the next. With optimization of the parameters involved in laser lysis, the sampling efficiency was found to be of the order of 60% under the best conditions. When compared with an independent sampling mechanism involving mechanical shearing of the cell using a capillary tip, in addition to application of a voltage gradient, the results were quite similar. This suggests that GFP sampling losses directly due to the laser pulse are minimal. Furthermore, this is corroborated by the

fact that direction of the laser pulse  $10\ \mu\text{m}$  laterally from the cell resulted in poorer sampling efficiencies than when it was directed under the centre of the cell. The lack of photodegradative effects observed within the cell, however, is not entirely surprising since the laser pulse is highly focused, and thus these effects may be highly localized at best to the very lowest margin of the cell, if not completely contained within the glass surface. Owing to the speed of this lysis technique, it is ideal for the study of highly dynamic processes in cells. As such this technique has been applied to investigate the activity of several important cell signalling kinases using enzyme-specific peptide reporters (Meredith *et al.* 2000; Li *et al.* 2001b, 2004). Additionally, Li *et al.* (2001a) was able to show that by modulating the laser pulse energy they could lyse portions of a single neuronal process without damaging the neuron itself, or using higher pulse energies they were able to sample small clusters of cells. This opens the possibility with precise tuning of laser parameters to selectively lyse single cells among groups of cells such as spherical aggregates or other three-dimensional tissue structures.

Laser lysis is highly suitable for integration into microfluidic chip platforms since it requires only optical access to the zone of cell lysis. As such it requires no extra channels or electrodes and thus does not increase the complexity of the chip design. Quinto-Su *et al.* (2008) have examined laser lysis on non-adherent BAF-3 cells in  $30\ \mu\text{m} \times 50\ \mu\text{m}$  channels in a polydimethylsiloxane (PDMS) chip using high-speed imaging. They found that in relation to laser-induced cavitation in a dish, significantly less energy from the laser pulse was transferred into cavitation bubble energy due to deformation of the PDMS walls. However, for single-cell studies, this is probably of very little consequence, since laser pulse power can be easily adjusted if needed, and the pulse energy required for plasma formation seems to be more than sufficient for lysis of a single cell. A larger problem arises in microfluidic chips from the predominant use of non-adherent cells in this format. Owing to the lack of attachment of the BAF-3 cells in the microfluidic channel, the cells that were not precisely centred with respect to the laser pulse were observed to be displaced rather than torn apart. Another complication that arose due to the chip format was the persistence of bubbles milliseconds to seconds after the laser pulse. Since bubbles can cause disruptions in fluid and electrical currents, this is undesirable for many post-lysis procedures. However, the authors of the study posited that these persistent bubbles were due to dissolved gas in either the PDMS, which could be remedied by coating the PDMS with a gas-impermeable coating, or in their buffer, which could be degassed. Despite these issues, they were able to show, using fluorescent images of cells loaded with a fluorescent dye, that after expansion of the cell contents during bubble formation, the cell contents were reconcentrated, leaving larger, slow diffusing species virtually undiluted. This is highly desirable for detection of low copy species, since maintaining high concentrations is essential for sensitive detection. Frequency-tripled Nd-YAG lasers (355 nm) have also been used to lyse single cells on microfluidic chips (He *et al.* 2005). In this



case, the cells were encapsulated within a picolitre-sized aqueous droplet. Laser lysis was performed inside the confined droplet such that the cell contents were mixed with an enzyme substrate so that enzyme kinetics could be followed. In this case, laser lysis allowed for a simple lysis mechanism since the lysis laser was coupled to the optical tweezer laser, as well as the fluorescence detection laser. This allowed the cell to be selected, held in place, lysed and the contents of the lysate analysed by fluorescence, without any further manipulations.

### 3. MECHANICAL LYSIS

Direct mechanical lysis using sharp surfaces with nanoscale barbs termed 'nanoknives' has also been recently demonstrated as a method to lyse single cells (Di Carlo *et al.* 2003). In this method, the cells were driven through a grating composed of 3  $\mu\text{m}$  wide serrated features, created using multiple isotropic deep reactive ion etch stages to form sharp ridges spaced vertically 0.34  $\mu\text{m}$  apart, separated by 3  $\mu\text{m}$  wide gaps that have been patterned onto a microfluidic chip. The cells run through an array of these nanoknives at a sufficient velocity are generally efficiently lysed. Some cells, however, stick to the upstream surface of the array and slowly elongate between the nanoknives eventually separated into several vesicles. Although this method led to 99% cell lysis according to a trypan blue quenching assay, measurements of free protein post lysis indicated that only 6% of total protein was freely released. The disparity between these numbers indicates that the majority of protein was recaptured within vesicles after lysis, or remained within cells that were merely porated as opposed to fully disrupted.

### 4. ACOUSTIC LYSIS

Sonication involves the use of ultrasonic waves to generate localized areas of high pressure resulting in cavitation that can shear apart cells. Sonication has several limitations that keep it from being widely used for single-cell lysis including the requirement of more than 50 s for the lysis of cells such as lymphocytes, which can result in significant heating and thus denaturing of proteins, and excessive diffusion of the cell contents that can lead to difficulties in downstream detection (Xue & Yeung 1996; Zhang & Jin 2004, 2006). However, when used after first treating the cell with a weak detergent such as digitonin, sonication can lyse cells within 3 s, allowing efficient separation of cell contents (Zhang & Jin 2004, 2006).

### 5. ELECTRICAL LYSIS

Electric fields generating transmembrane potentials of the order of 0.2–1.5 V cause rupture of the lipid bilayer forming pores, and with sufficient magnitudes of electric field strengths and time of exposure lead to cell lysis (Tessie 1992; Lu *et al.* 2006). The electric field strength required to reach the threshold to promote cell lysis will thus depend on cell size and shape, as shown in figure 2, as well as membrane composition (fluidity). The effect of size can be shown through comparative

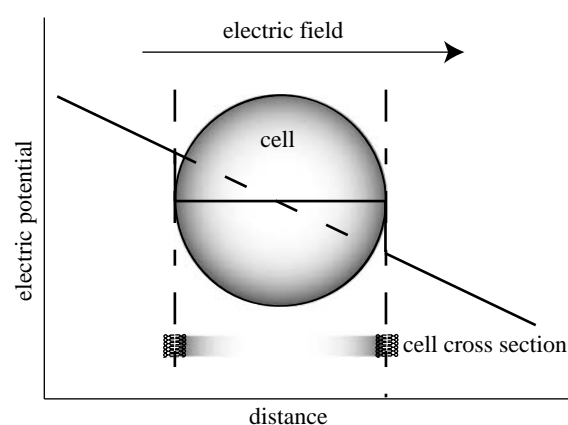


Figure 2. Schematic of voltage drops across the cell given an electric field. Solid line, through cell; dashed line, through buffer.

lysis of plant protoplasts (20–40  $\mu\text{m}$ ) versus microbes (1–2  $\mu\text{m}$ ), while a DC field of 7–10  $\text{kV cm}^{-1}$  is required to lyse the smaller microbial cells, the larger plant protoplasts are lysed using only 1.5–1.75  $\text{kV cm}^{-1}$  electric fields (Lee & Tai 1999). For use in capillary electrophoresis, Han *et al.* (2003) used gold-tipped capillaries and metal-coated glass slides as electrodes to create a voltage field vertically through adherent rat basophilic leukaemia (RBL) cells. After examining the voltage pulse length, magnitude and interelectrode distance, they found that they could achieve complete cell lysis in less than 33 ms using a 1 ms pulse length, at 40 V across a 20  $\mu\text{m}$  gap, yielding an electric field of 20  $\text{kV cm}^{-1}$ . An electric field of 20  $\text{kV cm}^{-1}$  represents a 2 V drop over the length of a 10  $\mu\text{m}$  cell, indicating that at the margins of the cell closest to the electrodes, there will be a 1 V drop across the cell membrane (assuming far greater electrical resistance across the membrane than through the cell cytoplasm, thus creating a constant potential in the cell interior). This is consistent with the values listed above for the voltage drop needed across the cell membrane for electrical lysis, as well as with the values determined by Lee & Tai (1999) for plant protoplasts and microbial cells. With this level of electric field, Han *et al.* (2003) showed that they were able to achieve similar levels of Oregon green sampling from single cells to those obtained using laser lysis. Although they noted the formation of bubbles on the electrodes using these conditions, they did not encounter problems from bubbles being injected into the capillary or peak broadening due to joule heating. Nashimoto *et al.* (2007) used a similar set-up, with a metal-coated slide and metal-tipped capillary tip as electrodes. They found that 10  $\mu\text{s}$  pulses were sufficient for cell lysis when using a 40 V drop and 20  $\mu\text{m}$  gap between electrodes. However, lysis was performed in a sugar solution as opposed to physiological buffer, and the requirement for speed of lysis was less stringent, requiring only that lysis occurred within 2 s of the pulse, since they were interested only in collecting RNA from the cell lysate, and not more temporally variable data such as enzyme kinetics. Furthermore, differences between the RBL cells and MCF-7 cells may have been another factor that allowed faster pulses to be sufficient.

Continuous mode microfluidic chips present a more challenging platform to provide effective electrical lysis conditions. The high-voltage drops needed to obtain irreversible poration of the membrane are sufficient to cause electrolysis of water, thus causing bubble formation that could interfere with subsequent sample handling techniques. Furthermore, if the electrodes are not placed within very close proximity (10's of  $\mu\text{m}$ ), joule heating could become a significant problem at the voltages required, especially since dissipation of heat is less effective in many microfluidic chip formats compared with free solution or round small diameter capillaries. To avoid these problems, Munce *et al.* (2004) used lower electric field strengths ( $300 \text{ V cm}^{-1}$ ), in combination with a narrowing of the separation channel, in order to bring about cell lysis in myeloid leukaemia cells of the order of 300 ms. Although this is clearly below the voltage drop threshold listed above for lysing single mammalian cells (approx.  $10 \mu\text{m}$  diameter), they posited that mechanical shear in co-operation with the electric field would lead to cell lysis. Microscopic observation revealed, however, that the cell membrane remained largely intact, keeping organelles and the majority of nucleic acid within the cell. They further examined the sampling efficiency of calcein from cells and found that 30% of the calcein fluorescence was drawn out of the cell towards the downstream detection point. Because the cell membrane was kept largely intact, it was assumed that the calcein was either in organelles that were not sampled and/or bound to proteins that were positively charged and thus migrated away from the detection area. McClain *et al.* (2003) used a different strategy to employ electrical lysis for serial analysis of single cells on a microfluidic chip. They employed a combination of AC and DC fields to keep from sustained high-voltage periods. As a result, the cell was exposed to peak voltages of approximately  $900 \text{ V cm}^{-1}$  for cell lysis, while in between pulses a voltage gradient of  $450 \text{ V cm}^{-1}$  was maintained for electrophoretic separation of the cell contents. AC cycling was optimized to 75 Hz since higher frequencies (more than 100 Hz) led to poor cell lysis, while lower frequencies led to peak broadening. Although the electric field used in this study was much lower than that used in the Han *et al.* (2003) study even at peak voltages, lysis was observed in less than 33 ms. This is likely due to the concurrent exposure of the cell to the hypotonic surfactant containing separation buffer that was mixed with the cell buffer as the cell approaches the separation channel. Several other studies have used similar techniques, employing a mix of electrical lysis with combinations of hypotonic detergent containing high or low pH buffers to generate rapid lysis at lower field strengths (Hogan & Yeung 1992; Xue & Yeung 1996; Gao *et al.* 2004).

## 6. CHEMICAL LYSIS

### 6.1. Detergent lysis

Detergent-based lysis arises from incorporation of detergent into the cell membrane, solubilizing lipids and proteins in the membrane, creating pores within

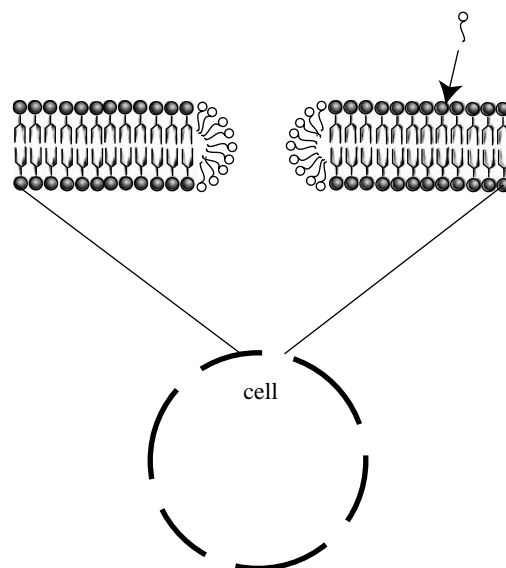


Figure 3. Schematic of poration and lysis of cell due to incorporation of detergent in the cell membrane.

the membrane and eventually full cell lysis (figure 3). Detergent lysis has been well developed for bulk biochemical assays and translates well into the single-cell level. Many different detergents are used for this purpose, including ionic, non-ionic and zwitterionic moieties. The selection of surfactant is significant since it can affect the speed of cell lysis, as well as the protein extraction efficiency. Strong ionic detergents such as sodium dodecyl sulphate (SDS) are able to provide cell lysis of the order of seconds, tending to denature proteins from the cell. This is advantageous for subsequent separation of neutral proteins by micellar electrokinetic capillary electrophoresis providing negative charge to the protein proportional to its molecular weight. However, it is less desired if the extracted protein is to be used in protein binding or enzyme activity assays. Milder non-ionic detergents such as Triton X-100 cause slower cell lysis, but have a much lower tendency to denature proteins and break up protein complexes and thus are preferable for applications involving protein structure or activity. Zwitterionic detergents such as 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulphonate can be used for cell lysis as well, and result in no net change in the charge of solubilized protein; however, care must be taken as they can result in the suppression or reversal of electroosmotic flow in downstream electrophoretic separation methodologies. The largest obstacle to applying detergent lysis for single-cell analysis is the ability to quickly deliver and mix the surfactant with the selected cell. By capillary electrophoresis, simple sequential loading of the cell followed by detergent-containing buffer represents the simplest solution. Although this requires time for diffusion of the detergent to the cell, this time can be minimized by pressure-induced injection to use the shape of the laminar flow profile to surround the cell laterally by lysis buffer and reduce the diffusion distance. Using this method, cell lysis can result after a 1 min mixing time with 0.1% Triton X-100 containing buffer (Berezovski *et al.* 2007). Another strategy for selecting a single cell

Table 2. Comparison of commonly used approaches for single-cell lysis.

lysis mode	adherent cells	suspension cells	temporal resolution	protein denaturation	specialized equipment
laser	+++	++	ns- $\mu$ s	none/low	yes
electrical	++	+++	$> \mu$ s	none/low	yes
detergent	+++	+++	$> s$	low/high	no

and subjecting it to detergent lysis is to use the capillary tip as a micropipette to pick up a single cell using pressure-based injection and to then deposit the cell in a micro-reactor vessel filled with lysis buffer. Using this method, Shoemaker *et al.* (2005) lysed single cells and were able to sequentially sample aliquots of the reaction mixture to follow the progress of enzyme assays.

For microfluidic chips, different mixing methods must be used. Ocivirk *et al.* (2004) used a Y-shaped microfluidic chip to mix a cell stream containing physiological buffer with a lysis buffer stream containing detergent and the substrate of the enzyme  $\beta$ -galactosidase. Because they were ultimately interested in enzyme activity within the cell, they chose the slower lysing 0.1% Triton X-100 (30 s) over the faster lysing 0.5% SDS (less than 2 s), in order to retain enzyme activity. However, uncertainty in the results of their enzyme assay due to the slow variable lysis times was added, which made it more difficult to determine the level and timing of mixing of the enzyme substrate with the intracellular components. Huang *et al.* (2007) provided an alternate solution, by trapping the cell using a pair of valves, and then filling the chamber where the cell was immobilized with lysis buffer containing fluorescent antibodies for labelling of proteins. Using this method, they were able to lyse a single cell and label intracellular proteins with a specific antibody employing 10 min incubation. This lysate was then subjected to electrophoretic separation and the antibody-bound proteins were quantified.

Marc *et al.* (2007) have developed a method whereby adherent cells can be analysed serially using detergent lysis followed by capillary electrophoresis. In this method, the cells are grown in a channel with constant flow of physiological buffer, and they are then sampled one by one by lowering the capillary over the most downstream cell. Electrophoretic buffer containing SDS is then introduced using sheath flow around the capillary inlet, which envelops the cell and is carried away downstream to the waste reservoir. Electrophoresis is initiated simultaneously so that cell contents as they are liberated are injected into the capillary. This procedure then can be carried out on the next most downstream cell that remains immersed in physiological buffer, for sequential sampling, allowing an analysis rate of one cell every 2 min.

## 6.2. Alkaline lysis

Another novel method for chemical cell lysis relies on the generation of  $\text{OH}^-$  ions at electrodes to create significant concentrations to drive cell lysis. Di Carlo *et al.* (2005) found that cells (HeLa, CHO, erythrocytes) could be lysed exclusively in proximity to the cathode by

application of a small electric field  $43 \text{ V cm}^{-1}$  between palladium electrodes. By comparing cell lysis results with simulations, they determined that lysis occurred in regions where electrolysis of the buffer would generate  $\text{OH}^-$  concentrations above 20 mM, which matched well with the limit of  $\text{OH}^-$  required to lyse cells tested by addition of NaOH.

## 7. CONCLUSION

There are many alternatives available for single-cell lysis, of which the three foremost methods currently are electrical, laser and detergent lysis (attributes summarized in table 2). However, depending on downstream applications, certain methods are preferable to others. The use of shear forces to rip apart the cell is quite attractive since it places no restrictions on buffer composition and thus can be easily used with physiological buffer to maintain the viability of the cell up until lysis. This will further allow proteins to remain in their native forms, enabling their use in downstream applications such as enzyme assays. Laser lysis due to the speed of the lytic process is especially promising for assays that require high temporal resolution. It is particularly well suited for analysis of adherent cells or settled suspension cells, since this lysis mechanism requires the cell to be at a specific focal height to be located in the zone of lysis. However, using cell capture strategies (Di Carlo *et al.* 2006), or hydrodynamic focusing techniques, it should be possible to accurately position suspension cells for reproducible lysis. Electrical lysis is also capable of high-speed lysis of single cells, though, except in the case where the cells are grown directly on an electrode, sequential lysis of adherent cells poses a significant challenge due to buffer electrolysis. These problems are exacerbated by the difference in magnitude of electric fields needed for lysis versus electrophoretic separations that commonly follow lysis, making continuous serial injections very difficult. Suspension cells, however, are easily manipulated using pressure-induced flow into channels where electrical lysis can occur. Similar to optical lysis mechanisms, electrical lysis imposes very few restrictions on the buffer to be used, although buffers with higher ionic strengths will ultimately lead to greater generation of joule heat, which could pose some problems. Chemical lysis ultimately depends on the diffusion of the lysis chemical to and throughout the cell, and thus is a relatively slow lysis technique. Although this diffusion can potentially be rendered faster by heating, sonication, convective flow or application of electric fields, it still remains difficult to generate fast enough mixing to obtain low millisecond to sub-millisecond lysis times in a controllable manner.



Since chemical lysis relies on the properties of the buffer to lyse the cell, it can thus present limitations for the selection of buffer composition. The presence of detergents in the buffer may have positive consequences including the solubilization of membrane proteins and reduced aggregation of proteins. However, it can also have undesired effects such as denaturation of proteins and alteration of protein separation properties. Detergent lysis is, however, a highly economical method of lysis and represents an excellent method for proof of concept studies, as no expensive equipment such as high-voltage power supplies or pulsed lasers are required.

Selection of single-cell lysis methodology will depend on the precise application that it is used for, with each of the main lysis techniques occupying its niche in the application domain. Further tuning and characterization of the parameters, which affect membrane poration and/or rupture, using these techniques will lead to more elegant ways to selectively release and/or lyse organelles within single cells, increasing our ability to measure and manipulate on subcellular levels. Furthermore, certain elements of the lysis method presented here such as poration of cells to introduce molecules (for drug or gene delivery) into cells or the use of cell lytic chemicals to improve electrophoretic separations can also be exploited in multiple capillary electrophoresis and lab-on-a-chip technologies.

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